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Natural Penetration Enhancers and Nanocarriers for Advanced Transdermal Drug Delivery: From Mechanisms to Clinical Translation

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Abstract

TDDS, which offer consistent drug release, good patient compliance, no first-pass effect, and few adverse reactions compared to oral and parenteral administrations, have attracted considerable interest as innovative TDDS. However, due to the densely organized arrangement of the SC, the most crucial barrier of effective transdermal transport is restricted delivery of drug through skin. NPEs including terpenes, essential oils, fatty acids and alkaloids are extensively studied owing to biocompatibility, low toxicity, and reversibly influencing the permeation of drug across skin lipids. Simultaneously, various nanotechnology-based DDS such as liposomes, transfersomes, ethosomes, niosomes, nanoemulsions, SLNs, NLCs were explored to overcome inherent problems in TDDS like poor drug solubility, stability, poor control of release, and improper targeting to the skin. Natural penetration enhancers along with the nanotechnology-based DDS offer a synergistic approach for significantly increasing drug flux, deposition, residence time, and bioavailability, as well as for decreasing skin irritation. This review summarizes structure and permeability of skin barrier; types and mechanisms of natural enhancers; main types of nanoscale carriers for TDDS; formulations and characterization techniques; safety evaluation methods; clinical considerations. The future breakthroughs for designing advanced formulation through AI assisted formulation design, artificial skin in 3D printed form, personalized medicine-based concept, and hybrid nanocarrier physical systems will greatly help the next-generation transdermal system. The combined application of natural enhancers and nanotechnology represents a promising avenue for the development of safe, effective, and patient-centric transdermal therapeutic systems.

Keywords

Natural penetration enhancers, Nanocarriers, Transfersomes, Ethosomes.

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1. INTRODUCTION:

Many of the problems and drawbacks associated with different routes of administration have led to the development of various drug delivery technologies. The conventional oral forms like tablets and capsules which are most frequently used due to their ease of administration and patient compliance are associated with poor bioavailability, gastrointestinal degradation of drugs, pre-systemic metabolism, gastric irritability, unpredictable absorption, etc. Parenteral administration offers very rapid delivery and therapeutic response, but is typically invasive, associated with pain, risk of infection and can only be performed by experienced personnel. This led to the need of other alternate means for drug delivery like TDDS. TDDS represents

the largest market in the global passive drug delivery field because it allows direct introduction of drugs into the systemic circulation thereby avoiding first-pass effect of the liver and also gives controlled and extended release of drugs. It also facilitates reduced dosage frequency, easy removal in case of emergency and better patient compliance. [1-5]

Commercial examples of transdermal therapeutic systems such as nicotine patches, fentanyl patches, clonidine, estradiol and scopolamine patches are available. The skin, while the largest and most accessible surface area, poses a challenge to successful transdermal administration due to its efficient barrier function. The outer barrier is formed by an outmost layer called the stratum corneum, which plays a vital role in limiting the passage of aqueous soluble compounds across the skin barrier including hydrophilic drugs, vaccines, proteins and peptides etc. Thus, for effective use of the skin, overcoming skin barrier is important to transport the drugs across. [6-10] Several techniques of transdermal enhancers include, use of penetration enhancers either chemically, physical or carrier mediated approaches. Chemical penetration enhancers, e.g. dimethyl sulfoxide (DMSO), azone, alcohol, surface-active agents, and pyrrolidinones have shown promise, but there is concern for skin irritation, allergy, erythema and cell damage with prolonged or repeated application to the skin. These issues encourage development of safe and biologically compatible skin enhancers. [11, 12]

Natural penetrants are considered promising candidates for improving percutaneous absorption due to their safety, low toxicity and ready availability. Various terpenes (limonene, alpha-pinene), essential oils (mentha oil, ginger oil), fatty acids (oleic acid, caprylic acid) polysaccharides and alkaloids from different sources have demonstrated their use as enhancing agents. These compounds interact with stratum corneum in different mechanisms to improve drug permeation, like increasing lipids fluidity, disturbing the packing of lipids and enhancing hydration which may leads to increase and deeper penetration of drugs into skin tissues. In conjunction with this, nanotechnology offers several advantages including good drug loading and enhanced bioavailability.

Nano lipid carriers (NLCs), Solid lipid nanoparticles (SLNs), Nanoparticle dispersions, liposomes, transferosomes etc. have showed their potential for delivering therapeutic agents. Therefore, combination of natural penetration enhancers and nanoparticles provides synergy towards enhanced and effective permeation across skin barrier. Hence this review provides an overview about the use of natural penetration enhancer along with nanotechnology based transdermal system for efficient and sustained drug delivery. [13-16]

2. SKIN BARRIER AND DRUG PERMEATION:

Skin is one of the most complex organs in the body. Apart from performing other complex functions such as regulating body temperature, the skin serves as an important protective barrier against excess loss of body water and entry of foreign substances into the body.

2.1 Structure of the skin:

The human skin is broadly divided into three main functional and morphological compartments namely epidermis, dermis and hypodermis.

a. Epidermis:

This is the outermost layer of the skin and comprises mainly the keratinocytes. The epidermis is made of five stratified cellular layers viz. stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum, each layer having a distinct physical and biochemical property which is important to determine the fate of drugs when apply topically.

b. Dermis:

This layer lies beneath the epidermis and is characterized by loose and dense irregular connective tissues and is rich in components such as collagen, reticular fibers, fibroblasts, mast cells, macrophages etc. It houses blood vessels, lymphatic vessels, hair follicles, sebaceous glands, sweat glands and nerves. When drugs permeate epidermis, it can enter the blood in dermal vessels for systemic circulation.

c. Hypodermis:

Also known as subcutaneous tissue, this is the innermost layer composed primarily of fat cells (adipose tissue). The fat in this layer supports a wide variety of other structures such as nerve endings, larger blood vessels, and lymphatics.

Among the all-layer epidermis is the chief contributor to impede drug transport across the skin.

2.2 Stratum corneum as the primary barrier:

The stratum corneum, is composed of dead or degenerated keratinocytes, called corneocytes, which are tightly packed in many layers and embedded in an extracellular lipid matrix which are lipid bilayer of cholesterol, ceramides and fatty acids, these layers of corneocytes and their surrounding lipid domains collectively referred to as "Brick and mortar" structural arrangement constitute major functional skin barrier which prevents the passage of most of water-soluble compounds from outside into the body across the skin. The stratum corneum generally allow the penetration of those compounds

having appropriate physico-chemical properties; such as molecular weight < 500 daltons, logarithm of partition coefficient (Log P) between 1-3, some moderate polarity for dissolution into intercellular lipids but then partitioning into aqueous phase, and sufficient drug potency to produce the desired therapeutic effect when a smaller amount has permeated. Ideally skin enhancer used in TDDS should interact with stratum corneum, inducing a temporary alteration in barrier function and enhancing diffusion while causing minimal or no lasting damage or discomfort to the skin. [7-10]

2.3 Drug permeation routes:

Drug is permitted to travel across skin via a series of steps which require a molecule to permeate from its donor medium through five different regions. Drugs may enter systemic circulation via any of three general pathways into skin; (i) transcellular pathway, (ii) intercellular pathway, and (iii) appendageal pathway.

(i) Transcellular pathway:

The drug molecule must partition into the outer most corneocytes and pass across it, from its aqueous medium into lipid membrane and again into the cytoplasm to permeate out through the inner layer of corneocyte where the drug partition in to outer medium to allow for diffusion into outer medium and then pass through various skin layers. Since corneocytes are mainly consist of lipids. Thus to use transcellular route effectively drugs will be ideally highly permeable.

(ii) Intercellular pathway:

The drug is partitioned into the extracellular lipid matrix which are the regions surrounding the cells and then it would be permeated through them to outer region. The highly structured organization of the lipid matrix significantly hinders the movement of hydrophilic drugs but more readily supports the movement of highly lipophilic and amphiphilic drug molecules. Most skin enhancers will take effect on this pathway and improve the permeability of drug through their effects on lipids phase like altering packing arrangement, increase lipid fluidity, and increase amount of free fatty acids and phospholipids.

(iii) Appendageal pathway:

The third pathway involves penetration through the pilosebaceous units or sweat pores, the structure known as appendage. Appendages constitute less than 1% surface area of human skin. Though appendages offer a relatively lower barrier compared to stratum corneum, its small surface area is not sufficient for high delivery rate of drugs to the systemic circulation. [8-10]

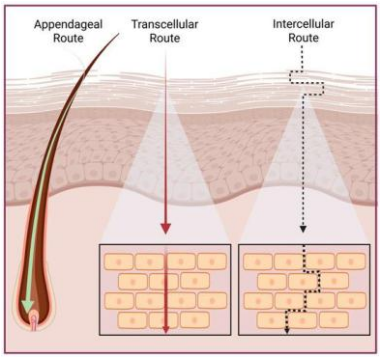


Figure 1: Routes of drug permeation through skin [17]

3. NATURAL PENETRATION ENHANCERS:

Natural penetration enhancers are compounds that come from biological sources and help drugs move through the skin while keeping the barrier intact. There's been a lot more interest in these substances over the past decade, mainly due to worries about the irritation and toxicity that can come with synthetic enhancers. Plus, more consumers are leaning towards products that use naturally derived ingredients, which have sped up their use in pharmaceutical and cosmetic formulations.

These natural enhancers come with a mix of chemical structures and mechanisms that let them boost the transdermal delivery of both hydrophilic and lipophilic drugs. Their multifunctional properties often bring added therapeutic benefits beyond permeation enhancement. [12-18]

3.1 Classification of natural penetration enhancers:

a) Terpenes:

Terpenes are the most studied group of natural penetration enhancers. These compounds are key components of essential oils and are classified based on the number of isoprene units in their structure.

Common terpenes include menthol, limonene, cineole, nerolidol, farnesol, pinene, and carvone. Many studies show they can improve transdermal transport by interacting with lipids in the stratum corneum and increasing drug diffusion. Compared to traditional enhancers like azone and DMSO, many terpenes have better safety profiles and are less likely to cause irritation. [13, 14]

b) Essential oils:

Essential oils are complex mixes of volatile plant chemicals from aromatic plants. Examples include peppermint oil, eucalyptus oil, clove oil, tea tree oil, and orange oil. These oils contain various bioactive compounds, including terpenes and terpenoids, which help enhance permeation.

In addition to improving skin permeability, essential oils often have antimicrobial, antioxidant, anti-inflammatory, and pain-relieving effects. These multifunctional properties make them appealing ingredients for modern transdermal formulations. [12]

c) Fatty acids:
Fatty acids are some of the oldest and most widely used penetration enhancers. Oleic acid, linoleic acid, lauric acid, and stearic acid have been used in several topical and transdermal products.
Oleic acid is especially effective because its unsaturated structure disrupts lipid packing in the stratum corneum. This disruption creates temporary micro domains that help improve the diffusion of therapeutic agents. Fatty acids are often used in Nano emulsions, lipid nanoparticles, and transdermal gels because they work well with lipid-based delivery systems.[11, 18]

Table 1: Classification of natural penetration enhancers

Class	Examples	Source	Primary mechanism	Applications
Terpenes	Menthol, Limonene, Cineole	Essential oils	Lipid fluidization	TDDS, invasomes
Essential oils	Peppermint oil, Eucalyptus oil	Plant extracts	Barrier disruption	Topical and transdermal systems
Fatty acids	Oleic acid, Linoleic acid	Vegetable oils	Lipid disordering	Nanoemulsions, gels
Alkaloids	Piperine, Capsaicin	Black pepper, chili	Partition enhancement	TDDS and phytopharmaceuticals

d) Alkaloids and other phytochemicals:
Bioactive alkaloids like piperine and capsaicin have shown promising properties for enhancing permeation. These compounds can change lipid organization, improve drug partitioning, and boost local blood flow. Many phytochemicals also have their own pharmacological effects that may work together to improve therapeutic results. Recent studies have also looked at flavonoids, polysaccharides, sterols, and plant-derived phenolic compounds as possible penetration enhancers. Their various mechanisms of action provide chances to design multifunctional transdermal systems that can enhance both permeation and treatment effectiveness.

3.2 Mechanisms of action of natural penetration enhancers:
Natural penetration enhancers enhance the transdermal transport of therapeutics by involving a variety of reversible and synergistic effects that result in changes to the stratum corneum without irritation or damage. Mechanisms of action are governed by chemical characteristics such as the molecular structure, lipophilicity, molecular size and affinity of compounds towards lipids and proteins of the skin. Major mechanism include the disruption of skin lipids, enhancing partitioning of the drug, modifications of keratine, increasing skin hydration, and enhancing penetration through the follicular route.

a) Lipid disruption and fluidization:
Many natural enhancers exhibit the tendency to disrupt the stratum corneum structure through fluidization of the intercellular lipids (e.g. Ceramides, cholesterol, fatty acids). Substances such as menthol, limonene, cineole and oleic acid can interact with these lipids, resulting in an increase in mobility and disorder of the lipid bilayer to facilitate diffusion through the formed lipid pores. For example, oleic acid is shown to form micelle or cavity that serve as paths through stratum corneum lipid barrier for hydrophilic and lipophilic drugs as well.

b) Enhancement of drug partitioning:
Apart from modifying the lipid barrier, natural permeation enhancers are also used to enhance the solubility and partition coefficient of the drug between vehicle and skin. Certain essential oils constituents and alkaloids enhance the partition of the drug to the lipids of stratum corneum, thereby increasing the solubility of drug molecules within the stratum corneum lipids and thereby increasing their thermodynamic activity.
For example piperine, a bioactive alkaloids isolated from black pepper, significantly improves permeation by increasing drug affinity towards biological membranes.

c) Keratin conformational modification:
Certain natural permeation enhancers may alter the conformational structure of the keratin protein to allow formation of intracellular spaces and thus to increase the availability of permeation channels. This approach provides additional paths in addition to intercellular pathways that enhance the drug permeation either transcellular or intercellular pathways.

d) Enhanced skin hydration:
Increased water content on the surface can enhance skin permeation by swelling of corneocytes, as well as widening intercellular space.

Increased hydration reduces diffusional barrier and some natural enhancers may act as humectants that increase water retention in the skin and enhance permeation.

e) Increased follicular transport:

Follicle pathways are an important channel for drug penetration and accumulation. Skin penetration and accumulation through the follicle pathway is also improved by natural permeation enhancers through increasing the drug solubility within the sebum, decreasing interfacial tension or altering composition of sebum. [18]

4. Nano-enabled transdermal drug delivery systems:

Nanotechnology has opened new avenues for percutaneous drug delivery, especially for the development of improved carrier systems that can surmount biological barriers and enable greater drug penetration through skin. Nanocarriers have outstanding physicochemical features such as ultra-fine particle size, vast surface area, high drug-loading capacity and modifiable surface groups which result in better drug solubility, modulated release and site-specific distribution.

The development of transdermal systems using nanotechnology has broadened the scope of molecules that can be delivered via skin to include poorly soluble drugs, peptides, proteins phytoconstituents and macromolecular therapeutics. [19, 20]

4.1 Liposomes:

Liposomes are spherical vesicles formed by the assembly of phospholipid bilayer. The phospholipid molecules form bi-layer membrane structure with aqueous (water-soluble) compartments in-between. Liposomes can encapsulate water-soluble and water-insoluble agents because of their amphiphilic nature. Liposomes have similar composition as of biological membranes and are highly biocompatible and less toxic.

When applied to the skin, liposomes enhance the localization of the drug within the epidermis and dermis. Conventional liposomes can show limited penetration beyond these layers of the skin due to their relatively rigid nature, but still are good vehicles for topical treatment requiring local delivery. [21]

4.2 Transfersomes:

Transfersomes are elastic vesicular carriers that have been developed to overcome the deficiencies of liposomes. Transfersome vesicle consists of phospholipids along with an edge activator (surfactant) that give unprecedented flexibility to the vesicle membrane.

The ultra deformability of transfersomes allows them to cross intercellular constrictions ranging from 3-10 times smaller than them. Transfersomes, propelled by hydration gradients across the skin, can travel deep into epidermal and dermal tissues. This results in increased systemic absorption. Transfersomes have better deformability than liposomes which makes them advantageous for poorly permeable drugs. [21]

4.3 Ethosomes:

The ethosomes are vesicles of modified phospholipids, in which high concentrations of ethanol are incorporated. The incorporation of ethanol facilitates two processes; it enhances the fluidity of vesicularly membranes and, at the same time, it alters the lipid structure of the stratum corneum.

The synergistic effect of the phospholipids and ethanol enables the ethosomes to reach the deeper skin layers more efficiently than conventional liposomes. Several studies have confirmed the superior transdermal delivery characteristics of various anti-inflammatory, anticancer, antifungal and cardiovascular drugs using ethosomal delivery systems. The improved penetration of ethosomes and their stabilizing effect has resulted in their being one of the most studied nanocarriers for transdermal drug delivery. [22, 23]

4.4 Niosomes:

Niosomes are vesicular systems based on nonionic surfactants and cholesterol. They are more chemically stable with less expensive production process and highly resistance against oxidation in comparison to liposomes.

The surfactant components make contact with the skin lipids and improve membrane permeability, but vesicle morphology offers a prolonged release of the drug. Niosomes have been shown to be effective vehicles for delivering anti-inflammatory drugs, anti-microbial agents, hormones and herbal bioactive. The ease of scale-up and the cost-effectiveness of niosomes have attracted interest for commercial development. [24]

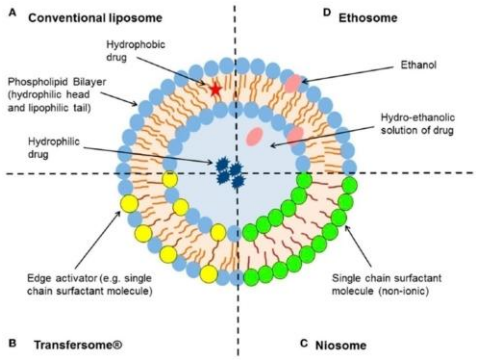


Figure 2: Major transdermal drug delivery nanocarriers

4.5 Nano emulsions:

Nano emulsions are thermodynamically or kinetically stable colloidal disperse system of oil, water, surfactants and co-surfactants, with droplet sizes usually in the range of 20 to 200 nm. Due to the extremely small droplet size, they have a greater interfacial surface area allowing for close contact with the skin.

Nano emulsions contribute to the increase of drug solubility and thermodynamic activity, as well as to the diffusion through the stratum corneum. Nan E is especially suitable carriers for drugs and herbal bioactive substances with poor water solubility. Besides, easy preparation characteristics and other patient friendly features increase their attractiveness for the formulation of transdermal delivery systems. [25]

4.6 Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs):

SLNs are being the first generation of lipid nanocarriers. They consist of entirely physiologically compatible solid lipids that are stable at room and body temperature, offering drug release control, stability and chemical protection.

However, the drug loading capacity of these SLNs is very limited due to their highly ordered crystalline structure. To address this issue, NLCs have been prepared by mixing solid and liquid lipids, thus producing a less ordered internal structure capable of incorporating higher amounts of drug molecules. NLCs show better loading capacity, less drug expulsion on storage, higher skin deposition and extended release. On this basis, it is one of the most promising lipid-based carrier systems suitable for transdermal and dermal delivery. [20, 26]

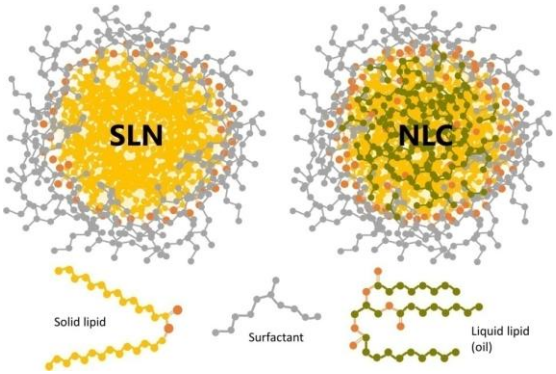


Figure 3: Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) [26]

Table 2: Advantages and limitations of nanocarriers

Carrier	Advantages	Limitations
Liposomes	Biocompatible	Limited deep penetration
Transfersomes	Highly deformable	Formulation complexity
Ethosomes	Excellent permeation	Ethanol-related instability
Niosomes	Stable, economical	Moderate loading efficiency
Nanoemulsions	High solubilization	Physical instability
SLNs	Controlled release	Limited drug loading
NLCs	High loading capacity	Complex optimization

5. Synergistic role of natural penetration enhancers in nanoformulations:

The interaction of natural penetration enhancers with nanotechnology provides a very promising approach to bypass the challenging barrier features of skin. Rather than working alone, these approaches exemplify synergism due to the complementary mode of action between these two topics that benefit drug penetration, retention and effectiveness.

The natural enhancers mainly alter the physicochemical properties of stratum corneum while the benefit of the nanocarrier is to enhance the drug solubilization, protection, targetability and release profile. The combined present of two approaches together will show synergetic results which were overcome for each approach used separately.

Natural penetration enhancer
+
Nanocarrier
↓
Skin lipid fluidization
+
Increased drug solubility
+
Enhanced skin hydration
+
Controlled drug release
↓
Higher flux across skin
↓
Enhanced bioavailability
↓
Improved therapeutic outcome

Figure 4: Synergistic action of natural penetration enhancers and nanocarriers [27]

NPE-loaded nanoemulsions:

The researchers found that nanoemulsions, which incorporated terpenes-limonene, menthol and cineole-proved outstanding permeation enhancements. The effect can be explained as the interaction of the terpene with the intercellular lining, which affects the extra-cellular cascade, coupled with the fact that the nanoemulsions keep the drug at the highest solubility possible. Therefore, such condition greatly raises the drug flux and bioavailability.

NPE-based ethosomes and transfersomes:

Invasomes are a relatively recent innovation in vesicular drug delivery technology. Invasomes consist of phospholipids, ethanol and natural terpenes in an all-in-one carrier system. The presence of vesicle deformability combined with the lipid fluidization effects of ethanol and permeation enhancement effects of natural terpenes on the skin structure facilitates great penetration.

Multiple studies demonstrate remarkably increased drug deposition in the skin, along with greater systemic drug absorption, from invasomal formulations versus conventional liposomes and ethosomes.

NPE-containing SLNs and NLCs:

Furthermore, the addition of the natural enhancer to lipid nanoparticles may have some extra benefits. Fatty acids and terpenes included in NLC matrix can increase both penetration and drug retention at the same time. Lipid nanoparticle creates an occlusive film on the skin surface, which reduces transepidermal water loss and increases hydration. The natural enhancer transports across the stratum corneum.

Enhanced permeation and drug retention:

Naturally derived penetration enhancers in conjunction with nanocarriers synergize can be attributed to a number of clinically significant advantages, such as an increase in transdermal flux of drug, enhanced skin deposition, increased residence time resulting in prolonged drug retention, enhanced systemic bioavailability, decreased frequency of administration, and improved compliance by patients. [28-31]

Table 3: Representative studies on natural penetration enhancer-based nano-formulations

Drug	Nanoformulation	Natural enhancer	Outcome
Temoporfin	Invasomes	Terpenes	Improved dermal penetration
Raloxifene HCl	Invasomes	Limonene	Enhanced bioavailability
Various lipophilic drugs	Ethosomes	Cineole	Increased permeation
Anti-inflammatory drugs	Nanoemulsions	Menthol	Higher skin flux
Multiple therapeutic agents	NLCs	Oleic acid	Improved retention and controlled release

6. Formulation and characterization strategies:

That systematic, scientific formulation design is crucial to bioavailability enhancement, nano-enabled transdermal formulations formulating effective means to utilize nano formation technology. A number of different factors have

potential to affect performance of the systems. These include particle size, particle charge, lipid composition, surfactant level, drug load and enhancer level.

6.1 Formulation approaches:

Choice of an appropriate nanocarrier would depend on the physiochemical characteristics of the drug, therapeutic goals, target tissue, and the required release profile. Natural penetration enhancers could be either entrapped in the lipid phase, the aqueous phase, or the interfacial region of the delivery system depending upon their propensity and mode of action.

The concentration of the enhancer has to be carefully considered. If concentrations are too low the enhancement of permeation may not be significant and high concentrations may destabilize the nanocarriers or cause irritancy of the skin.

Adoption of Quality by design (QbD) concepts in modern formulation development is growing continuously. This approach follows a fundamental sequence initiated by the preparation of the Quality target product profile (QTPP) followed by delineation of Critical quality attributes (CQA's), Critical material attributes (CMA's) and Critical process parameters (CPP's). Many statistical designed experiments (e.g. Box-Behnken design, Central composite design, factorial design) are used commonly for the evaluation of the interactions of variables to achieve the best formulation.

Common formulation variables are lipid concentration, amount of surfactant and co-surfactant, drug loading efficiencies, amount of enhancer, ethanol percentage, speed of homogenizer, time of sonication and temperature of process. The choices of all these variables help for the formulation stability, reproducibility, permeability characteristics and for scale up processes. [32]

6.2 Characterization of nano-enabled TDDS:

Careful physicochemical and biological characterization of the developed nano-enabled transdermal drug delivery systems (TDDS) are required during development because therapeutic outcomes are directly influenced by various characteristics of nanocarriers including the rate and extent of drug loading, stability, penetration, release behavior and biocompatibility. Hence, the extensive use of modern characterization tools under both in vitro and in vivo conditions is necessary for established correlation between formulation characteristics and biological behavior.

a) Particle size and polydispersity index:

Particle size is one of the important parameters in development of nano-enabled TDDS, since small size with high surface to volume ratio facilitates better contact with stratum corneum, enhances diffusion and thus skin penetration through intercellular spaces as well as follicular routes. Researchers revealed that particles having size less than 300nm were better with skin permeation.

The distribution of particle size within Formulation is assessed using PDI. Higher PDI signifies broad distribution of sizes or presence of aggregates; whereas lower PDI indicates mono dispersity or uniform size distribution within Formulation. A PDI value of formulation ranges between 0.1 and 0.3 is desired as indicating physical stability and mono dispersity. [33]

b) Zeta potential:

Zeta potential is the measure of surface charge and it determines the electro-chemical stability of formulation system which influences interactions between particles. Generally, nanoparticles carrying zeta potential of more than 30 mV are regarded stable because of mutual repulsion between particles to prevent aggregation. Skin interactions of charged particles were reported.

Positive zeta potential enhances more skin penetration and adherence because of electrostatic interactions with the negatively charged skin. Although negative charge may retard skin absorption due to repulsion, positive charges can leads to skin irritation. [34]

c) Entrapment efficiency and drug loading:

The entrapment efficiency of any formulation relates to its capacity to incorporate a therapeutic agent within formulation system; higher the entrapping efficiency of formulation higher would be the retention of drug in the formulation systems for longer duration and results into sustained and more efficient drug delivery system, reduced drug wastage and consequently greater therapeutic benefits. The entrapment efficiency depends on formulation-based parameters like drug solubility, the concentrations of lipids or surfactant used in formulation, methods used in preparation etc.

Drug loading capacity determines amount of therapeutic agent contained in a fixed amount of formulation material; this value should be high to deliver therapeutic dose of drug with a limited dose of the formulation. NLC formulation has been able to deliver more drug loading compare to conventional SDDs and other DS.

d) Morphological evaluation:

Morphology is concerned with shape of the formulation system which plays significant role on biological behavior. Transmission electron microscopy, scanning electron microscopy, atomic force microscopy etc are used to study shape and

surface features of nanoparticles/nanovesicles which help determine structure, size and physical stability of formulation.

e) *In Vitro* drug release studies:

This study is designed to analyze release profiles of a drug from various carrier systems through biological barrier or surrogate membrane *in vitro*. Dialysis membrane-based study for drug release from FDDS, SDDDS is used for *in vitro* assessment.

Models like first order, zero order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas model are used to predict release kinetics and mechanism of drug release.

f) *Ex vivo* skin permeation studies:

These studies give a direct measure of ability of formulations to enhance the penetration of drug across a biological membrane. The most popular method is to use a standard Franz diffusion cell. Different species like animals (rodents like mouse, rat) or skin from human cadavers can be used to carry out these experiments. Various types of skins including the porcine skin which closely simulates human skin have been experimented.

In vitro permeation experiments measures parameters such as: permeability co-efficient, lag time, drug flux and enhancement ratio.

g) Skin retention and visualization studies:

Beyond a direct assessment of the penetration performance of nanoparticles/ vesicles/ systems, these measurements of actual drug distribution patterns in different layers of skin tissue.

Techniques such as confocal laser scanning microscopy (CLSM), fluorescence microscopic, multi-photon microscopy, and Raman spectroscopy are extensively used for real-time monitoring and distribution mapping of the drugs within the skin tissue layers for gaining knowledge of delivery pathways and fate of nano-enabled TDDS. [35, 36]

7. SAFETY ASSESSMENT AND REGULATORY CONSIDERATIONS:

While natural penetration enhancers have been traditionally thought of as safer to use than synthetic chemical alternatives, they still require thorough safety assessments when used in nano-enabled transdermal delivery systems. The constraints imposed by nanoparticle application (e.g. Small particle size and large surface area) may change biological interactions and hence the long-term safety. Regulatory agencies tend to focus on toxicity testing during development of the product.

7.1 Skin irritation and sensitization:

Skin irritation remains a concern for applications of transdermal formulations. Excessive disruption of the stratum corneum lipids may lead to erythema, edema, dryness, itching, and inflammation. Although relative to the synthetic enhancers such as dimethyl sulfoxide, terpenes, fatty acids, and essential oils tend to induce less skin irritation, concentration-dependent adverse effects have been known to occur.

Numerous reliable tests are available to determine the degree of irritation potential. The Draize skin irritation test has traditionally been a popular choice, although there are other, validated experiments which are no less reliable and are now promoted as equivalent options. OECD test guideline 404 deals with internationally accepted procedures used to access acute dermal irritation, while the Human repeat insult patch test (HRIPT) remains a valuable practice used in clinical trials to test for sensitization potential. [37-39]

7.2 Cytotoxicity assessment:

In vitro cytotoxicity tests give initial indications of cell compatibility and any possible toxicological concerns. Human keratinocyte and fibroblast cell lines are used because they are representative of the major cell types in skin.

There are several assays that are used to effectively evaluate cell viability after treatment with nanoformulations. The MTT assay is the most commonly used and there are several other assays which have been adopted to assess membrane integrity, oxidative stress, apoptosis, production of pro-inflammatory cytokines and mitochondrial activity.

7.3 Histopathological evaluation:

The histopathological evaluation offers insight to the structural changes produced within the skin tissues subsequent to treatment. On the microscopic level, interpretation of features can include epidermal thickening, cell shape, infiltrates of inflammation and changes in the distribution of lipids.

For a successful penetration enhancer, it is believed that temporarily offing the structure of stratum corneum, without causing irreversible damage to tissue, is more desirable. Histology study thus becomes very important to differentiate reversible modification of barrier from tissue harm.

7.4 Regulatory challenges:

The regulatory environment for nano-enabled transdermal products is still complex and developing. Some problems in the assessment process will arise, especially in the evaluation of formulations using penetration enhancers derived from natural sources. Natural products have a built-in variability from their geographic origin, cultivation system, harvesting, and extraction processes. Chemical composition and biological activity may vary among preparations. In addition, characterization of nanoparticles is still a

technical challenge, as colloidal systems are constantly changing.

Regulatory agencies are expected to demand a great deal of physicochemical characterization, stability information, toxicological data and manufacturing controls prior to acceptance. Providing detailed information on the properties of the nanoparticles and lifetime safety considerations will be necessary for regulatory submittals. [40-41]

Table 4: Safety profile of common natural penetration enhancers

Enhancer	Source	Irritation potential	Safety status
Menthol	Peppermint oil	Low to moderate	Widely accepted
Limonene	Citrus oils	Low	Generally safe
Cineole	Eucalyptus oil	Low	Generally safe
Oleic acid	Vegetable oils	Low	Well established
Piperine	Black pepper	Moderate at high concentrations	Requires optimization
Capsaicin	Chili pepper	Moderate to high	Dose dependent

8. Clinical translation and future perspectives:

However, despite much progress in the field of formulation science and optimistic results achieved during preclinical trials, relatively little progress has been made in translating nano-enabled transdermal drug delivery systems clinically. Transitioning these novel formulations from bench to bedside remains one of the greatest hurdles still facing the field.

8.1 Current clinical status:

A number of transdermal products have been tremendously successful in the marketplace. These include patches delivering nicotine, fentanyl, lidocaine, estradiol, rivastigmine and clonidine. Although most of the products approved to date fall into the standard transdermal segment,

If these are used in preclinical formulations supplemented with natural penetration enhancers there has been an increasing trend toward better permeation, higher bioavailability, longer duration of effect, increased skin retention and patient compliance. So far, however, few systems have advanced toward clinical assessment, and well-designed human studies are still required.

8.2 Manufacturing and scale-up challenges:

To achieve commercialization, manufacturing processes need to be established that consistently produce formulations having reproducible quality parameters. Achieving and maintaining the particle size, drug loading, encapsulation efficiency and physical stability of nanoparticles during scale-up fabrication is non-trivial and other worries such as storage stability, sterilization, packing compatibility, cost efficiency and GMP should be thought about. These problems' solutions are necessary to promote the industrial application and approval. [42-44]

8.3 Emerging trends:

A number of promising new technologies are anticipated to revolutionize transdermal drug delivery by overcoming the drawbacks of traditional formulations and thus, enhancing the delivery efficiency. In this context, the use of artificial intelligence (AI) and machine learning (ML) algorithms that are increasingly being adopted in the pharmaceutical R&D sector have been explored through a number of recent publications in Scopus- and Web of Science- indexed journals. Accordingly, it is shown that AI algorithms present can effectively predict drug-excipient compatibility, the optimization of nanocarrier composition, permeation properties, and stability of the formulation. Such computational approaches significantly reduce formulation development time and facilitate the rational design of advanced transdermal systems.

Another important advancement is the application of three-dimensional (3D) skin models. These engineered skin constructs closely replicate the structural, biochemical, and physiological characteristics of human skin, providing a more reliable platform for evaluating drug permeation, toxicity, and formulation performance. Compared with conventional cell cultures and animal models, 3D skin models offer improved predictability of clinical outcomes and support the growing emphasis on reducing animal experimentation in pharmaceutical research.

The concept of personalized transdermal therapy is also gaining considerable attention in the era of precision medicine. Future transdermal formulations may be tailored according to individual patient characteristics, including genetic background, skin physiology, age, disease condition, and metabolic profile. Such patient-centric approaches have the potential to improve therapeutic effectiveness while minimizing adverse effects and interindividual variability.

Furthermore, hybrid systems integrating nanocarriers with physical enhancement techniques such as microneedles, iontophoresis, electroporation, and sonophoresis represent a

promising direction for future research. These combined approaches can significantly enhance drug permeation and enable the transdermal delivery of challenging molecules, including peptides, proteins, vaccines, and nucleic acid-based therapeutics. Collectively, these emerging technologies are expected to expand the clinical applicability of transdermal drug delivery and contribute to the development of safer, more effective, and patient-oriented therapeutic systems. [45-47]

9. Conclusion:

Natural penetration enhancers have gradually emerged as potential substitute for synthetic permeation enhancers due to the excellent safety profiles, bio-degradability and multitasking biologic activities. Terpenes, essential oils, fatty acids and alkaloids can readily alter barrier properties of the SC through different strategies, such as lipid disruption, enhanced hydration, drug partitioning and follicular transportation. Similarly, nanocarriers (e.g., liposomes, transfersomes, ethosomes, nanoemulsions, SLNs and NLCs) significantly enhanced permeability of drugs, their loading efficiency and retention ability, as well as their targeted delivery and sustained release performance in the skin. Therefore, co-incorporation of NPEs and nanocarriers can enhance permeation efficiency, skin deposition and residence, bioavailability of the drugs and prolong release duration compared with NPE alone or combined nanocarriers alone. Despite the remarkable preclinical development, challenges regarding the formulation and characterization of NPE-loaded nanocarriers, large-scale manufacturing of these products, the regulations on their commercial approval and the safety evaluation with longer clinical follow-ups still pose hurdles for the translation of nanotechnology based TDDS from bench to clinic. Future advances like AI assisted drug formulation design, advanced 3D skin model with enhanced permeability properties, personal medicine for different skin characteristics and hybrid physical-nanocarrier systems are likely to accelerate the translational of next-generation transdermal therapies. Overall, nano-enabled transdermal systems incorporating natural penetration enhancers represent a highly promising strategy for achieving safe, efficient, and clinically translatable drug delivery.

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