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Nanoparticles for Dermal and Transdermal Drug Delivery

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1. Introduction

The term “nanoscale” refers to particle size range from ~ 1 to 100 nm [1], but for the purpose of drug delivery, nanoparticles in the range of 50 – 500 nm are acceptable depending on the route of administration. The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived and concentrations above or below this range can be toxic or produce no therapeutic benefit. The slow progress in the efficacy of the treatment of several diseases has suggested a growing need for a multidisciplinary approach to the delivery of therapeutics to target tissues [2]. Transdermal drug delivery systems (TDDS) or patches are controlled-release devices that contain the drug either for localized treatment of tissues underlying the skin or for systemic therapy after topical application to the skin surface [3]. TDDS are available for a number of drugs, although the formulation matrices of these delivery systems differ. They differ from conventional topical formulations in the following ways:

- they have an impermeable occlusive backing film that prevents intensive water loss from the skin beneath the patch;
- the formulation matrix of the patch maintains the drug concentration gradient within the device after application so that drug delivery to the interface between the patch and the skin is sustained; and
- TDDS are kept in place on the skin surface by an adhesive layer ensuring drug contact with the skin and continued drug delivery [4].

Topical or transdermal drug delivery is challenging because the skin acts as a natural and protective barrier. TDDS were introduced into the US market in the late 1970s [5], but transdermal delivery of drugs had been used for a very long time. There have been previous reports about the use of mustard plasters to alleviate chest congestion and belladonna plasters as analgesics. The mustard plasters were homemade as well as available commercially where mustard seeds were ground and mixed with water to form a paste, which was in turn used to form a dispersion type of delivery system. Several methods have been examined to increase the permeation of therapeutic molecules into and through the skin and one such approach is use of nanoparticulate delivery system.

The skin has been an important route for drug delivery when topical, regional, or systemic effects are desired. Nevertheless, skin constitutes an excellent barrier and presents difficulties for the transdermal delivery of therapeutic agents, since few drugs possess the characteristics required to permeate across the stratum corneum in sufficient quantities to reach a therapeutic concentration in the blood [6]. In order to enhance drug transdermal absorption, different methodologies have been investigated, developed, and patented. Improvement in physical permeation-enhancement technologies has led to renewed interest in transdermal drug delivery. Some of these novel advanced transdermal permeation-enhancement technologies include iontophoresis, electroporation, ultrasound, microneedles to open up the skin, and more recently the use of transdermal nanocarriers.

2. The human skin

The potential of using the intact skin as the port of drug administration to the human body has been recognized for several decades. However, the skin is a very difficult barrier to the ingress of materials allowing only small quantities of a drug to penetrate over a period of time. In order to design a drug delivery system, one must first understand the skin anatomy and its implication of drug-of-choice and method of delivery.

The human skin is the largest organ in our body with surface area of 1.8-2.0 m². It is composed of three main layers; the epidermis, dermis and hypodermis (subcutaneous layer) (Fig. 1). The skin is a well energized organ that protects the organism against environmental factors and regulates heat and water loss from the body.

3. Routes of drug penetration through the skin

The permeation of drugs through the skin involves the diffusion through the intact epidermis through the skin appendages (hair follicles and sweat glands). These skin appendages form shunt pathways through the intact epidermis, occupying only 0.1% of the total human skin. It is known that drug permeation through the skin is usually limited by the stratum corneum (Fig. 2). Three main penetration routes are recognized (Fig. 3).

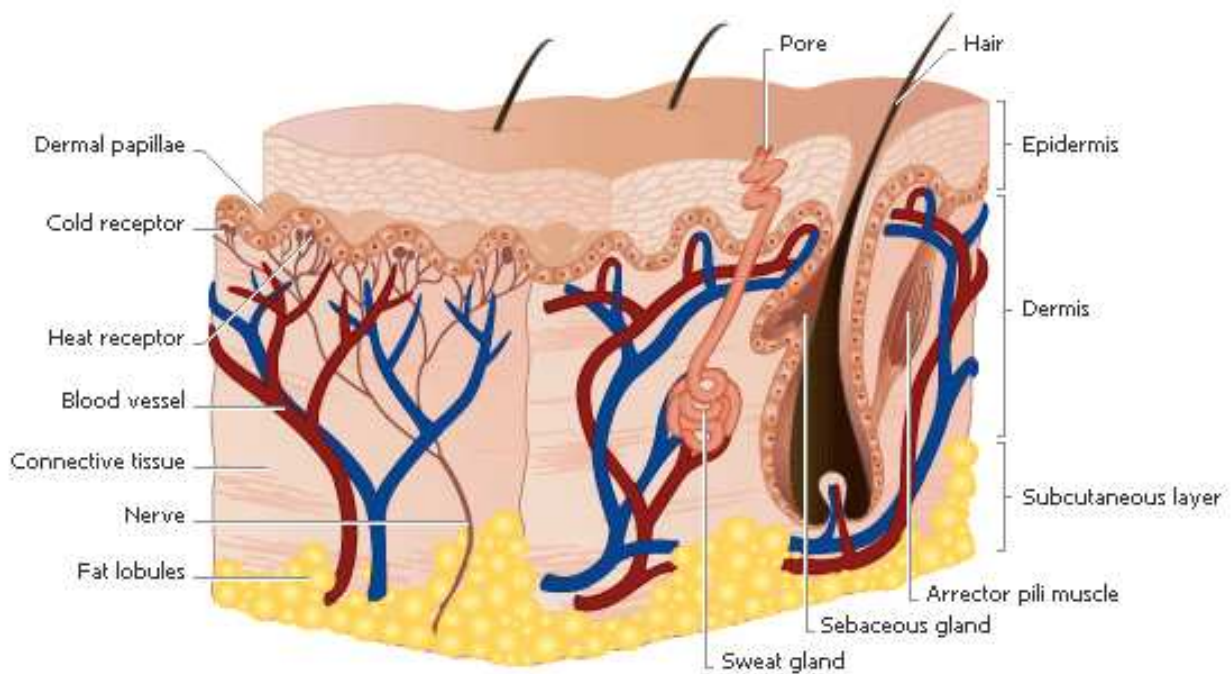
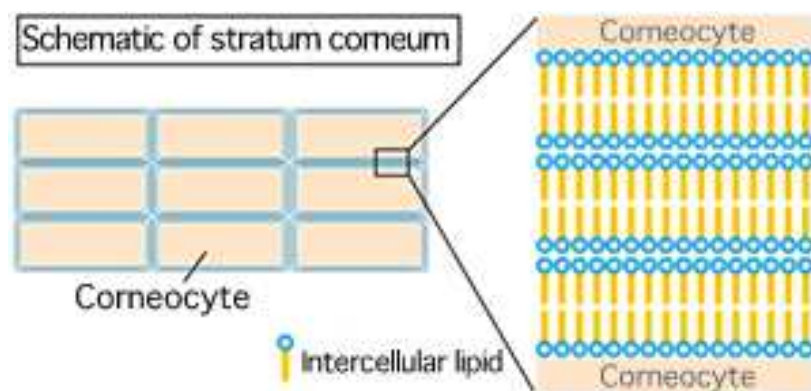


Figure 1. Structure of the skin (<http://www.naturrussia.com/natural/skin/structure.html>. Downloaded on April 26, 2014)



(http://www.spring8.or.jp/en/news_publications/press_release/2011/110406/. Downloaded April 26, 2014).

Figure 2. The stratum corneum

3.1. The intercellular lipid route

Interlamellar regions in the stratum corneum, including linker regions, contain less ordered lipids and more flexible hydrophobic chains. This is the reason for the nonplanar spaces between crystalline lipid lamellae and their adjacent cells' outer membrane. Fluid lipids in skin barrier are crucially important for transepidermal diffusion of the lipidic and amphiphilic molecules, occupying those spaces for the insertion and migration through intercellular lipid

layers of such molecules [7]. The hydrophilic molecules diffuse predominantly “laterally” along surfaces of the less abundant water-filled interlamellar spaces or through such volumes; polar molecules can also use the free space between a lamella and a corneocyte outer membrane to the same end.

3.2. The transcellular route

Intracellular macromolecular matrix within the stratum corneum abounds in keratin, which does not contribute directly to the skin diffusive barrier but supports mechanical stability and thus intactness of the stratum corneum. Transcellular diffusion is practically unimportant for transdermal drug transport [8]. The narrow aqueous transepidermal pathways have been observed using confocal laser scanning microscopy. Here, regions of poor cellular and intercellular lipid packing coincide with wrinkles on skin surface and are simultaneously the sites of lowest skin resistance to the transport of hydrophilic entities. This lowest-resistance pathway leads between clusters of corneocytes at the locations where such cellular groups show no lateral overlap. The contribution to transdermal drug transport can increase with pathway widening or multiplication, e.g., that which is caused by exposing the stratum corneum to a strong electrical (electroporation/iontophoresis), mechanical (sonoporation/sonophoresis), or thermal stimulus, or suitable skin penetrants.

3.3. Follicular penetration

Recently, follicular penetration has become a major focus of interest due to the fact that drug targeting to the hair follicle is of great interest in the treatment of skin diseases. However, follicular orifices occupy only 0.1% of the total skin surface area. For this reason, it was assumed to be a nonimportant route for drug penetration. But a variety of studies have shown that hair follicles could be an interesting option for drug penetration through the skin [6]. Such follicular pathways have also been proposed for topical administration of polystyrene nanoparticles. They were investigated in porcine skin (*ex vivo*) and human skin (*in vivo*). Surface images revealed that polystyrene nanoparticles accumulated preferentially in the follicular openings. This distribution was increased in a time-dependent manner, and the follicular localization was favored by the smaller particle size. The study also confirmed similarity in the penetration between both membranes (porcine and human skin). In other investigations, the influence of microparticle size in skin penetration has been shown by differential stripping. Nanoparticles can act as efficient drug carriers through the follicle or can be utilized as follicle blockers to stop the penetration of topically applied substances.

4. Main factors for nano-based delivery system

4.1. Particle size, size distribution and zeta potential

Particle size and shape affect drug release, physical stability and cellular uptake of the nanoparticulate materials. The yield and size distribution of each system are affected by certain

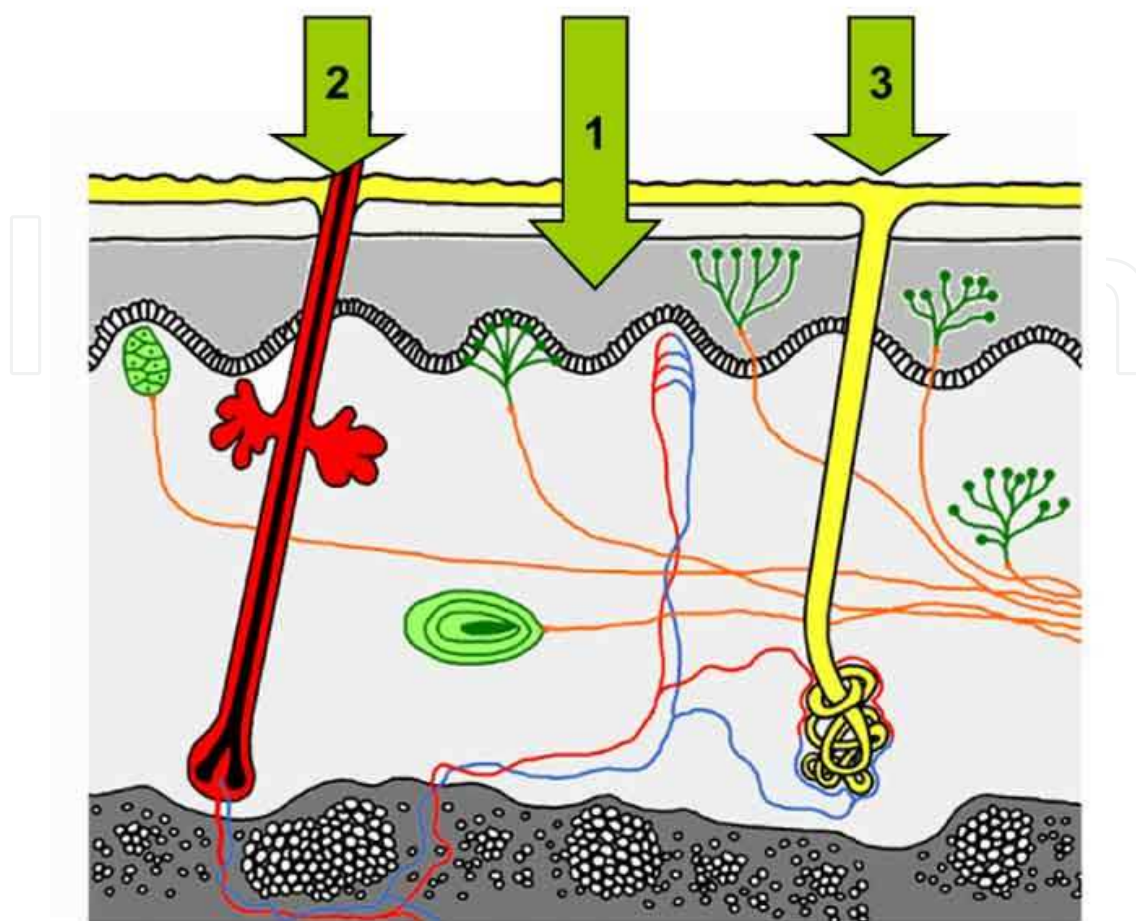


Figure 3. Structure of the skin showing routes of penetration: (1) across the intact horny layer, (2) through the hair follicles with the associated sebaceous glands, or (3) via the sweat glands (<http://www.skin-care-forum.basf.com/en/author-articles/strategies-for-skin-penetration-enhancement/2004/08/12?id=5b9a9164-6148-4d66-bd84-6df76bd6d111&mode=Detail>. Downloaded April 26, 2014).

in-process operations and conditions such as stirring rate, temperature, type and amount of dispersing agent as well as the viscosity of the organic and aqueous phases [9,10]. Zeta potential of a dispersion is necessary for dispersion stability [11].

4.2. Surface properties

The attachment of nanoparticles to cell membrane is affected by the surface charge of the particles. Variation of the particle surface charge could potentially control binding to the tissue and direct nanoparticles to cellular compartments both *in vitro* and *in vivo*. Cellular surfaces are dominated by negatively charged sulphated proteoglycans molecules that play pivotal roles in cellular proliferation, migration and motility [12]. Cell surface proteoglycans consist of a core protein anchored to the membrane and linked to one or more glycosaminoglycan side chains (heparan, dermatan, keratan or chondrotine sulfates) to produce a structure that extends away from the cell surface.

Nanoparticles show a high affinity for cellular membrane mainly due to electrostatic interactions [12]. It is known that cell membranes have large negatively charged domains, which should repel negatively charged nanoparticles. The high cellular uptake of negatively charged nanoparticles is related first to the non-specific process of nanoparticles adsorption on the cell membrane and second to formation of nanoparticle clusters [13]. The adsorption of the negatively charged particles at the positively charged sites via electrostatic interaction can lead to localized neutralization and a subsequent bending of the membrane favouring in turn endocytosis for cellular uptake [14]. Thus the formulation of nanoparticles with different surface properties can influence their cellular uptake and intracellular distribution and it is possible to localize the nanoparticles to specific intracellular targets (lysosomes, mitochondria, cytoplasm, etc) by modifying their surface charge [15].

There are some investigations that showed the effect of surface charge, for example polymer charge density of dendrimers was found to significantly impact membrane permeability. The most densely charged polymer facilitates the transport of dye molecule across the membrane [16]. Other investigation showed that lipid coating of ionically charged nanoparticles was able to increase endothelial cell layer crossing 3 or 4 fold compared with uncoated particles, whereas nanoparticles coating of neutral particles did not significantly alter their permeation characteristics across the endothelial cell monolayer [13]. Transdermal drug administration systems have been limited to certain drugs of a range of molecular weight and lipophilicity, and of certain charge preference. For instance, cationic compounds have a positive effect on skin permeation, since the skin carries a negative surface charge due to phosphatidylcholine [17] and carbohydrates found in mammalian cells contain negatively charged groups. Therefore, nanoparticles with predominant positive charge would promote transdermal permeation.

5. Dermatopharmacokinetics

Dermatopharmacokinetics describe the pharmacokinetics of topically applied drugs in the stratum corneum with pharmacodynamic effects. The smart techniques (tape stripping and microdialysis) use in dermatopharmacokinetic methodology assesses the cutaneous drug concentration at the site of application. Various studies have shown dermatopharmacokinetics to be a reliable and reproducible method for determining bioequivalence, and have indicated that it is applicable for all topical dermatological drug products. Dermatopharmacokinetics refer to the determination of stratum corneum concentration-time curves for topical actives. This is analogous to plasma/urine concentration-time curves for systemically or orally administered drugs, and the concept is clearly adaptable to microdialysis, where drug is determined in the skin compartment in which the microdialysis fibre is positioned (Fig. 4).

Although, this procedure is invasive, it is a method of great potential offering information of high value and relevance. There could be sampling in a compartment within the skin. It is a technically demanding procedure, however, requiring experimental dexterity of high order. The potential for use on diseased skin is a unique and considerable advantage over other techniques, but real challenges remain with respect to reproducibility, sensitivity, applicable drugs, etc.

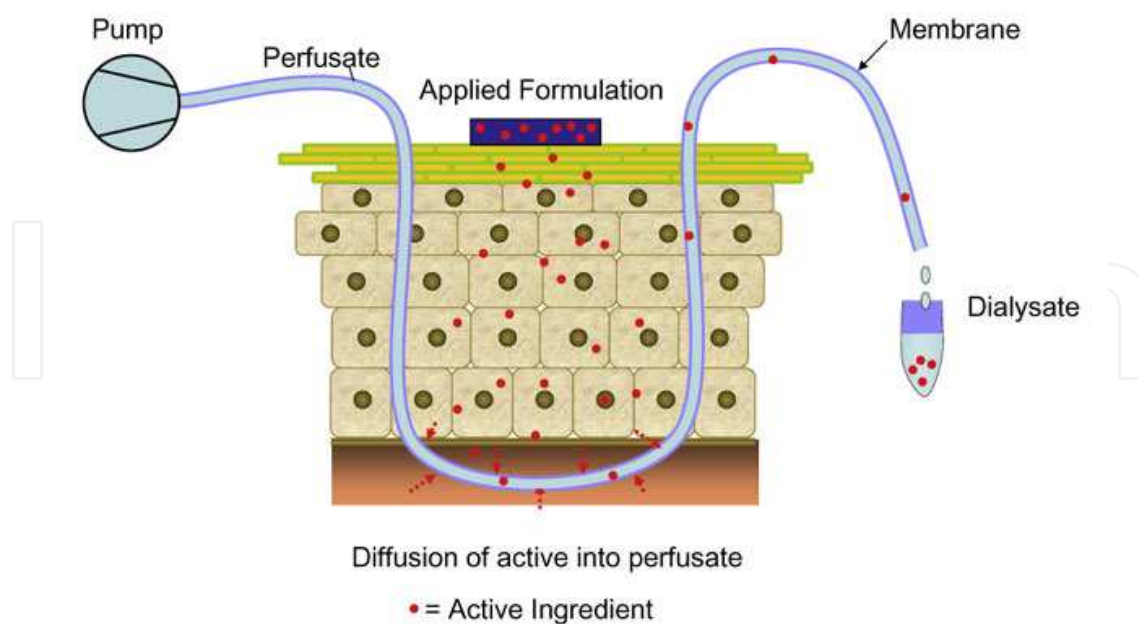


Figure 4. Sampling in the skin by microdialysis (<http://www.skin-care-forum.basf.com/en/author-articles/strategies-for-skin-penetration-enhancement/2004/08/12?id=5b9a9164-6148-4d66-bd84-6df76bd6d111&mode=Detail>. Downloaded April 26, 2014)

Stratum corneum tape-stripping is a minimally invasive method for determining drug levels in human stratum corneum *in vivo*. It involves repeated application of adhesive tapes on a site that has been treated with a topical formulation and determination of drug levels in stratum corneum collected on tape strips.

The dermatopharmacokinetics approach suggested by the Food and Drug Administration (FDA) proposes to evaluate the level of a topically applied drug in the stratum corneum during its uptake and clearance so as to calculate classic pharmacokinetic parameters [18]. The assumption is that stratum corneum concentration-time curves are directly related to concentration-time curves in the epidermis and dermis.

When applied to diseased skin, topical drug products induce one or more therapeutic responses, where onset, duration, and magnitude depend on the relative efficiency of three sequential processes, namely:

- the release of the drug from the dosage form
- penetration of the drug through the skin barrier, and
- generation of the desired pharmacological effect.

Because topical products deliver the drug directly to or near the intended site of action, measurement of the drug uptake into and drug elimination from the stratum corneum can provide a dermatopharmacokinetics means of assessing the bioequivalence of two topical drug products [19,20]. Presumably, two formulations that produce comparable stratum corneum concentration-time curves may be bioequivalent, just as two oral formulations are judged

bioequivalent if they produce comparable plasma concentration-time curves. Even though the target site for topical dermatologic drug products in some instances may not be the stratum corneum, the topical drug must still pass through the stratum corneum, except in instances of damage, to reach deeper sites of action [21]. In certain instances, the stratum corneum itself is the site of action. For example, in fungal infections of the skin, fungi reside in the stratum corneum and therefore dermatopharmacokinetic measurement of an antifungal drug in the stratum corneum represents direct measurement of drug concentration at the site of action [22]. In instances where the stratum corneum is disrupted or damaged, *in vitro* drug release may provide additional information toward the bioequivalent assessment. In this context, the drug release rate may reflect drug delivery directly to the dermal skin site without passage through the stratum corneum. For antiacne drug products, target sites are the hair follicles and sebaceous glands. In this setting, the drug diffuses through the stratum corneum, epidermis, and dermis to reach the site of action. The drug may also follow follicular pathways to reach the sites of action. The extent of follicular penetration depends on the particle size of the active ingredient if it is in the form of a suspension [21, 23-25]. Under these circumstances, the dermatopharmacokinetic approach is still expected to be applicable because studies indicate a positive correlation between the stratum corneum and follicular concentrations. Although the exact mechanism of action for some dermatological drugs is unclear, the dermatopharmacokinetic approach may still be useful as a measure of bioequivalence because it has been demonstrated that the stratum corneum functions as a reservoir, and stratum corneum concentration is a predictor of the amount of drug absorbed [26].

For reasons thus cited, dermatopharmacokinetic principles should be generally applicable to all topical dermatological drug products including antifungal, antiviral, antiacne, antibiotic, corticosteroid, and vaginally applied drug products. The dermatopharmacokinetic approach can thus be the primary means to document bioavailability/bioequivalence. Generally, bioequivalence determinations using dermatopharmacokinetic studies are performed in healthy subjects because skin where disease is present demonstrates high variability and changes over time. Use of healthy subjects is consistent with similar use in bioequivalence studies for oral drug products.

A dermatopharmacokinetic approach is not generally applicable when:

- a single application of the dermatological preparation damages the stratum corneum
- for otic preparations except when the product is intended for otic inflammation of the skin; and
- for ophthalmic preparations because the cornea is structurally different from the stratum corneum.

6. Ideal drugs for dermal and transdermal delivery

Owing to the selective nature of the skin barrier, only a small pool of drugs can be delivered systemically at therapeutically relevant rates [27]. Few drugs constitute the whole segment of

the transdermal drug market. Besides great potency, the physicochemical drug characteristics often evoked as favourable for percutaneous delivery include moderate lipophilicity and low molecular weight [28]. However, a large number of pharmaceutical agents do not fulfill these criteria. This is especially true for macromolecules, such as insulin, human growth hormone or cyclosporine, which are very challenging from the drug delivery point of view. The physicochemical properties of ideal drug for transdermal delivery include:

- Molecular weight less than approximately 1000 Daltons.
- Affinity for both lipophilic and hydrophilic phases. Extreme partitioning characteristics not ideal.
- Low melting point.
- Should be potent, with short half life and be non-irritating.

Overcoming low skin permeability to xenobiotics can be achieved by a variety of approaches, and is an active field of research. Their effectiveness and applicability will vary from drug to drug depending on the physicochemical nature of the compound. New drug discovery is still a complicated process and generally requires substantial time and monetary investment. Technologies for formulation change provide the benefit of improving pharmaceutical product efficacy and safety as well as patient convenience; these technologies provide a relatively simple approach to creating new pharmaceuticals compared with new drug discovery because the active compounds used in the formulation have already been approved [29-31]. Nnamani *et al* [32] developed and evaluated the antimicrobial activities of an alternative non-invasive, convenient and cost-effective transdermal drug delivery system (TDDS) containing gentamicin in biodegradable polyester-based matrices. Other drugs which have been formulated for dermal and transdermal delivery are nitroglycerin, nicotine, scopolamine, clonidine, fentanyl, 17- β -estradiol, testosterone, Boswellic acid (*Boswellia serrata*) and curcumin (*Curcuma longa*).

7. Advantages of dermal and transdermal drug delivery

Transdermal delivery provides convenient and pain-free self-administration for patients. It eliminates frequent dosing administration and plasma level peaks and valleys associated with oral dosing and injections to maintain constant drug concentrations, and a drug with a short half-life can be delivered easily. All this leads to enhanced patient compliance, especially when long-term treatment is required, as in chronic pain treatment and smoking cessation therapy [3,33,34].

- Avoidance of hepatic first-pass metabolism and the gastrointestinal (GI) tract for poorly bioavailable drugs is another advantage of transdermal delivery. Elimination of the first-pass effect allows the amount of drug administered to be lower, and hence, safer in hepato-compromised patients, resulting in the reduction of adverse effects.
- Transdermal systems are generally inexpensive when compared with other therapies on a monthly cost basis, as patches are designed to deliver drugs from 1 to 7 days.

- The other advantage of transdermal delivery is that multiple dosing, on-demand or variable-rate delivery of drugs is possible with the latest programmable systems, adding more benefits to the conventional patch dosage forms.
- The general acceptability of transdermal products by patients is very high, which is also evident from the increasing market for transdermal products.
- Transdermal route permits the use of a relatively potent drug with minimal risk of system toxicity [35,36].
- In case of toxicity, the transdermal patch can easily be removed by the patient [37].

8. Disadvantages of dermal and transdermal delivery systems

Even though dermal and transdermal delivery systems have a lot of advantages over conventional topical formulation, it still suffer from a lot of limitations. The disadvantages of dermal and transdermal delivery systems according to Ranade and Cannon [38] are that:

- Not all drugs are suitable for transdermal delivery.
- Drugs that require high blood levels cannot be administered.
- The adhesive used may not adhere well to all types of skin.
- Drugs or drug formulation may cause sensitization or irritation which must be evaluated fairly early in the development process.
- The patches may/can be uncomfortable to wear.
- The manufacture requires specialized equipments which results in the formulation being more expensive to manufacture than conventional dosage forms thus the formulation will not be economical for most patients.
- There is always a lag time for drug to penetrate through the skin to the systemic circulation, therefore TDDS is not suitable for drugs requiring rapid onset of action.
- There is a requirement for low dose/high permeable drug. In general a drug with molecular weight less than 400, $\log P_{o/w}=2-3$ and dose less than 10 mg will be the best candidate for transdermal delivery.

9. Characterization of dermal and transdermal delivery systems and their performance

Dermal and transdermal delivery systems are characterized using different methods.

9.1. Drug solubility determination

The determination of solubility of the drug in the transdermal/dermal matrix early in the formulation process can avoid crystallization problem, which is one of the instabilities in transdermal drug delivery systems (TDDS). This instability in the matrix which could be due to supersaturation makes the formulation metastable and upon storage results in changes in the liberation/release rate of the drug from the formulation.

9.2. Micromeritic measurements

9.2.1. Particle-size, shape and zeta potential analysis

Light scattering is an important way of characterizing colloidal and macromolecular dispersions and could be useful in assessing properties of particulate TDDS e.g. ethosomes. The particle size and size distribution are primarily measured using wet laser diffraction sizing otherwise called dynamic light scattering (DLS) [39]. Size of formulation can also be determined using dynamic light scattering (e.g. using a Zetasizer). This is necessary to ascertain the possible effect of the size on drug release and penetration across barriers in transdermal and dermal delivery as well as to monitor stability over time. The zeta potential of a formulation is very important. It is determined using Zetsizer or by other means, and gives information on the charge of the particles and the tendency of the particles in a formulation to aggregate or to remain discrete.

9.2.2. Specific surface area

An important parameter of bulk powders is the specific surface area expressed per unit weight. The specific surface area measurement includes the cracks, crevices, nooks, and crannies present in the particles. To include these features in the surface-area measurement, methods have been developed to probe these convoluted surfaces through adsorption by either a gas or a liquid [40-42]. The most widely used surface area measurement technique is the adsorption of a monolayer of gas, typically krypton or nitrogen as the adsorbate gas in helium as an inert diluent, using the method developed by Brunauer, Emmett, and Teller known as the BET method. Surface area affects spreading and occlusivity of TDDS.

9.3. Visualization by transmission electron microscopy

A combination of transmission electron microscopy (TEM) and freeze fracturing otherwise referred to as freeze fracture electron microscopy (FFEM) could be used to visualize skin structures and certain perturbations in the skin. A micrograph image is generated by transmitting a beam of electrons through a specimen appropriately treated to enhance the visualization of skin structural details. High resolution of TEM makes it possible to visualize both structures and transition processes in the epidermis. Using different techniques, epidermal granules [43], Langerhans cells [44] and the lipids in stratum corneum and epidermis [45], amongst others, have been observed. Samples preparation in FFTEM involves freezing the sample and subsequent longitudinal fracturing approximately parallel to the original skin

surface under high vacuum [46]. Further treatment could be done on the sample after which the fracture is viewed under high voltage. This visualization method can provide information on the interaction between the nanoparticle formulation and the skin. Since the fracture will always run along the plane of least resistance, FFEM micrographs of treated stratum corneum often show the lipid coated surfaces of corneocytes or the lipid lamellae.

9.4. Stability

Physical and chemical instabilities of carrier systems often limit their widespread use in medical applications [47]. Instabilities in ethosomes and other nanocarrier formulations are caused by hydrolysis or oxidation of the phospholipid molecules and are indicated by leakage of the encapsulated drug and alterations in vesicle size due to fusion and aggregation [48,49]. Changes in size and size distribution, entrapment efficiency and aggregation of vesicles are very important parameters in monitoring stability. These parameters can be assessed by EM or DLS repeatedly over time at varying storage conditions. It has recently been found that although multilamellar and large unilamellar benzocaine-loaded ethosome vesicles remained substantially stable with time, in terms of drug entrapment yield and particle dimensions, small unilamellar vesicles showed high tendency to form aggregates due to increased surface area exposed to the medium [10]. Such vesicle aggregation indicates instability. In addition, changes in storage conditions led to marked decrease in particle dimensions and drug-entrapment yield with less regular morphology for frozen-and-thawed multilamellar ethosome dispersions, while the untreated multilamellar and unilamellar vesicular dispersions remained homogenous and stable with regard to those parameters assessed over the period [50]. Temperature of formulation and storage conditions affect physical stability of nanoparticle preparations [10,51].

Optical characteristics, viscosity and physical changes such as cracking or creaming are also important in assessing stability of ethosomes. Ethosomes are colloidal disperse systems therefore, cracking and creaming may be observed during storage as in water-in-oil emulsions. The use of an innovative optical analyzer, Turbiscan Lab[®] Expert, in studying the influence of optical characteristics on long-term stability of vesicular colloidal delivery systems has been advocated [52]. The principle of this measurement is based on the variation of the droplet volume fraction (migration) or mean size (coalescence), thus resulting in the variation of backscattering and transmission signals as a function of time. No variation of particle size occurs when the backscattering profile is within the interval $\pm 2\%$. Variations greater than 10% either as a positive or negative value in the graphical scale of backscattering are representative of an unstable formulation.

9.5. High-pressure liquid chromatography (HPLC)

It is used to monitor the stability of pure drug substance and drugs in formulation with quantitation of degradation product. A liquid mobile phase is pumped under pressure through a stainless steel column containing particles of stationary phase with a diameter of 3-10 μm . The analyte is loaded onto the head of the column via a loop valve and separation of a mixture occurs according to the relative lengths of time spent by its components in the stationary phase.

All components in a mixture spend more or less the same time in the mobile phase in order to exit the column. The column effluent can be monitored with a variety of detectors.

The combination of high-pressure liquid chromatography (HPLC) with monitoring by UV/Visible detection provides an accurate, precise and robust method for quantitative analysis of pharmaceutical products and is the industry standard method for this purpose. The two principal mechanisms which produce retardation of a compound passing through a column are straight-phase packing where adsorption of polar groups of a molecule onto the polar groups of a stationary phase occur and reverse-phase packing which is due to partitioning of the lipophilic portion of a molecule into the stationary phase.

9.6. Liquid chromatography–mass spectrometry (LC/MS)

Mass spectrometry in conjunction with liquid chromatography provides a method for characterizing impurities in drugs and formulation excipients [53]. It provides highly sensitive and specific methods for determining drugs and their metabolites in biological fluids and tissues.

9.7. Fourier Transform infra red (FTIR) spectroscopy

FTIR spectroscopic properties are used to determine the chemical stability of the drug in a TDDS. FTIR spectra of formulations, the starting materials and pure drug sample are normally obtained at a range of 4000-400 cm^{-1} and the spectra obtained on infrared spectrophotometer using potassium bromide of spectroscopic grade.

Detailed insights into the organization of the stratum corneum can be gained through the study of the vibrations of amide, amine and carboxylic groups and the frequencies of the methylene stretching, scissoring and rocking vibrations. FTIR is used to study the lateral lipid organization of the intercellular lipid matrix in stratum corneum, which is essential for the barrier function of stratum corneum, as more densely organized membranes are less permeable to substances. The stretching vibrations are used to determine whether lipids are in an ordered (hexagonal or orthorhombic lateral packing) or disordered packing (liquid phase), while the scissoring and rocking vibration provide detailed information on the presence of orthorhombic phases. By performing measurements at different temperatures, also the thermotropic behaviour of the lipids can be determined.

9.8. Attenuated Total Reflectance FTIR (ATR-FTIR)

Attenuated total reflectance FTIR (ATR-FTIR) is a modification of FTIR. In this technique, IR radiation is not transmitted through the sample but reflected by the sample. With this technique, it is possible to perform measurements on stratum corneum *in vivo*, because the skin can be placed on the ATR crystal. The IR radiation beam penetrates only to a limited extent into stratum corneum. In order to detect substances in the stratum corneum, it is necessary to remove stratum corneum layers, by tape-stripping, which makes it also possible to generate a penetration profile of an applied substance in stratum corneum [54-56]. ATR-FTIR has been used to determine effects of topically applied substances on the lipid organization in the

stratum corneum [54,57]. ATR-FTIR can be combined with tape-stripping to determine the penetration profile of hydrophilic and lipophilic substances in stratum corneum in addition to the water profile of the stratum corneum.

9.9. Differential scanning calorimetry (DSC)

This technology is used to evaluate the degree of perturbation of the skin lipids as a result of penetration of a formulation or drug through skin. The free intercellular lipid bilayers of the stratum corneum have a unique composition compared to other epithelial lipid bilayers and consist of ceramides (50%), cholesterol (25%), and fatty acids (10-20%, highly enriched in linoleic acid). These common skin lipids are detected at different transition temperatures when the skin is subjected to DSC studies.

9.10. Small angle X-ray diffraction (SAXD)

This technique is used to analyse the long range order of the crystalline structure of lipids. Stratum corneum is a very thin layer of about 10 μm and composed of corneocytes and an intercellular lipid matrix. The ordered structure of the intercellular lipid matrix plays an important role in skin barrier function. Structural analyses of intercellular lipids in mammalian stratum corneum by X-ray diffraction have shown more detailed lipid structure models. The X-ray pattern of a lamellar phase is characterized by a series of sequential maxima, which are positioned at equal interpeak distances at increasing scattering angle [58]. The sequential peaks are referred to as the 1st order (positioned at distance Q_1), the 2nd order (Q_2), the 3rd order (Q_3), etc, in which Q is directly related to the scattering angle. The repeat distance (d) of a lamellar phase can be directly calculated from the peak positions $d=2\pi/Q_1=4\pi/Q_2=6\pi/Q_3$, etc. In skin research SAXD is used to study the lamellar organization of the lipids in the intercellular matrix of stratum corneum of humans and other mammals. Furthermore, SAXD measurements using lipid mixtures of ceramides, cholesterol and free fatty acids have revealed the role of the various lipid classes in the lamellar phases. Additionally, it has been used to study effects of topically applied substances [59] or physical stratum corneum perturbation methods [54]. SAXD is also used to study the effects of hydrophilic and lipophilic agents like nanoparticles on the lamellar organization of isolated stratum corneum.

9.11. Dermal irritation assay

If a new drug is intended to be applied to the skin or eyes, one of the first tests to be conducted would be to determine if the drug, or the formulation containing the drug, will cause irritation of the skin or eyes. Even if a drug is intended only for dermal application, eye irritation testing may also be required because of the possibility of inadvertent exposure to the eyes. The test is conducted as follows: Six male albino rabbits are to be clipped free of hair on the back. One area of skin is left intact, whereas another is abraded in a tic-tac-toe pattern with the point of a hypodermic needle so as to incise the superficial epidermis layer without causing bleeding. The test material, 0.5 ml of liquid or 0.5 g of solid or semisolid is applied to each site under a 1 \times 1 inch gauze pad. The entire trunk of the animal is wrapped with an impervious material and held in place with tape for 24 h. The patches are then removed and excessive material

wiped off. The skin reactions are scored at 24 and 72 h after the initial application according to a scheme such as that listed in Table 1.

Skin reaction	Value
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

Table 1. Dermal irritation scoring system

The mean values of the six rabbits for erythema and eschar formation at 24 and 72 h for both intact and abraded skin (four values) are added. The mean values of the six rabbits for edema at 24 and 72 h (four values) are also added. The total of eight values is divided by 4 to give the primary irritation index. Values of 5 or greater are considered indicative of a positive irritant [60].

9.12. Occlusivity

It is usually the aim of cosmetic chemists to maintain the skin's softness and freshness and it is considered important to retain moisture in the stratum corneum. The degree to which a formulation retains or promotes the loss of moisture from the stratum corneum is termed the occlusivity. The occlusivity of formulations for topical application is determined *in vivo* by measuring the suppression of transepidermal water loss (TEWL) of the skin. The occlusive effect of the formulation also depends on the characteristics of the skin such as the lipid level and prevailing environmental condition. The occlusivity of films formed by nanoparticles varies with time, type of formulation, coating amount, physical form, size of particles etc. It is necessary to determine the occlusivity of a nanoparticle formulation for topical application as it directly affects liberation and penetration of the encapsulated drug. Under occlusive conditions, the skin is more hydrated and transport of drug could be higher.

9.13. Spreadability

Pharmaceutical semisolid preparations include ointments, pastes, creams, emulsions, gels, and rigid foams. Their common property is the ability to cling to the application surface for a reasonable period of time before they are washed off or worn off [61]. They usually serve as vehicles for topically applied drugs, as emollients, or as protective or occlusive dressings, or they may be applied to the skin and membranes such as the rectal, buccal, nasal, and vaginal mucosa, urethral membrane, external ear lining, or the cornea [62]. These preparations are widely used as a means of altering the hydration state of the substrate (i.e., the skin or the mucous membrane) and for delivering the drugs (topical or systemic) by means of the topical–mucosal route. Nanoparticles for transdermal application could be formulated as gels, creams, emulsions, foams etc, or dispersed in ointment bases. This makes the spreadability characteristics of the formulation very pertinent in achieving the desired objective.

The efficacy of topical therapy depends on the patient spreading the formulation in an even layer to deliver a standard dose. The optimum consistency of such a formulation helps ensure that a suitable dose is applied or delivered to the target site. This is particularly important with formulations of potent drugs. A reduced dose would not deliver the desired effect, and an excessive dose may lead to undesirable side effects. The delivery of the correct dose of the drug depends highly on the spreadability of the formulation. Spreadability, in principle, is related to the contact angle of the drop of a liquid or a semisolid preparation on a standardized substrate and is a measure of lubricity, which is directly related to the coefficient of friction [63]. Spreadability is subjectively assessed at shear rates varying from 10^2 to 10^5 s^{-1} . The rate of shear during spreading, γ s^{-1} , is calculated using the following equation for plane laminar flow between two parallel plates:

$$\gamma = \frac{v}{d} \quad (1)$$

in which v is the relative velocity of the plates ($cm\ s^{-1}$) and d is the distance between them (cm); that is, a measure of thickness of the film between the skin surfaces [64].

To assess the spreadability of a topical or a mucosal semisolid preparation, the important factors to consider include hardness or firmness of the formulation, the rate and time of shear produced upon smearing, and the temperature of the target site [64]. The rate of spreading also depends on the viscosity of the formulation, the rate of evaporation of the solvent, and the rate of increase in viscosity with concentration that results from evaporation [65].

9.14. Rheology

Rheology is the science that studies how materials deform and flow under the influence of external forces. Characterization of the rheological properties of the system is important not only in the design of the product and its application, but during its processing and to ensure long shelf-life [66]. It is thus necessary to explore the rheological changes that our formulations would experience when subjected to external forces during manufacture and in use. To that effect, measurements of the shear stress, strain, viscosity are done on the formulations. This

property can also be used to assess the stability of the formulation over time. To obtain information about viscous and elastic behaviour as well as microstructure of the topical gels, flow viscometry, oscillatory rheometry, and transient measurements are conducted.

10. Novel technologies for dermal and transdermal application

Nanoparticles for dermatological applications such liposomes and other vesicular systems as well as other types of nanosized drug carriers such as solid lipid nanoparticles, nanostructured lipid carriers, polymer-based nanoparticles and magnetic nanoparticles have been developed. These have in one way or the other, addressed the shortcoming of the traditional TDDS such as ointments, gels etc. Different carrier systems have been proposed in an attempt to favor the transport of drugs through the skin, enabling drug retention and in some cases allowing a controlled release [6]. Skin penetration is essential to a number of current concerns, e.g. contamination by microorganisms and chemicals, drug delivery to skin (dermatological treatments) and through skin (transdermal treatments), and skin care and protection (cosmetics) [6].

Physicochemical properties of nanocarrier systems determine the interaction with biological systems and nanocarrier cell internalization. The main physicochemical properties that affect cellular uptake are size, shape, rigidity, and charge in the surface of nanoparticles. The most used and investigated nanocarriers for dermal/transdermal drug delivery in the pharmaceutical field include liposomes, transfersomes, ethosomes, niosomes, dendrimers, nanoparticles-lipid and polymer nanoparticles, and nanoemulsions. In general, the advantages and limitations of using nanocarriers for transdermal drug delivery are their tiny size, their high surface energy, their composition, their architecture, and their attached molecules. Table 2 summarizes the advantages and disadvantages of common transdermal nanocarriers.

10.1. Microemulsions

Microemulsions are dispersions with droplet size from 10 to 100 nm and do not have the tendency to coalesce [67-69]. Microemulsions form spontaneously with appropriate amounts of a lipophilic and a hydrophilic ingredient, as well as a surfactant and a co-surfactant [70]. Microemulsions have several specific physicochemical properties such as transparency, optical isotropy, low viscosity and thermodynamic stability [70,71]. As efficient drug carriers, microemulsions have been widely employed in both transdermal and dermal delivery of drugs [72,73].

Most of the microemulsions have very low viscosity, which may restrict their application to the transdermal delivery field due to inconvenient use [74]. The main mechanisms to explain the advantages of microemulsions for the transdermal delivery of drugs include the high solubility potential for hydrophilic drugs of microemulsion systems, permeation enhancing effect of the ingredients of microemulsions, and the increased thermodynamic activity of the drug in the carriers [68,70,71].

10.2. Nanoemulsions

Nanoemulsions are isotropic dispersed systems of two nonmiscible liquids, normally consisting of an oily system dispersed in an aqueous system, or an aqueous system dispersed in an oily system but forming droplets or other oily phases of nanometric sizes. They are thermodynamically unstable systems, in contrast to microemulsions, because some nanoemulsions need high energy to produce them. They are susceptible to Oswald ripening, and as a consequence susceptible to creaming, flocculation, and other physical instability problems associated with emulsions. Despite this, they can be stable (metastable) for long periods due to their extremely small size and the use of adequate surfactants. Hydrophobic and hydrophilic drugs can be formulated in nanoemulsions. They are nontoxic and nonirritant systems, and they can be used for skin or mucous membranes and parenteral and non-parenteral administration in general, and they have been utilized in the cosmetic field. Nanoemulsions can be prepared by three methods mainly: high-pressure homogenization, microfluidization, and phase-inversion temperature. Transdermal delivery using nanoemulsions has decreased due to the stability problems inherent to this dosage form. Some examples of drugs using nanoemulsions for transdermal drug delivery are gamma tocopherol, caffeine, plasmid DNA, aspirin, methyl salicylate, insulin and nimesulide [75].

Presently, transdermal nanoemulsion formulations are not developed as much as nanoparticles or liposomes due to the stability problems inherent to this dosage form. Nevertheless, gamma tocopherol, caffeine, plasmid DNA, aspirin, methyl salicylate, insulin, and nimesulide have been included in nanoemulsions. The use of these nanocarriers to deliver analgesics, corticosteroids, anticancer agents, etc, is very important, as these drugs are able to act immediately because they do not need to cross extra barriers [76-82].

10.3. Vesicular systems

10.3.1. Liposomes

Liposomes (Fig. 5) are spherical, self closed vesicles of colloidal dimensions, in which phospholipid bilayer sequesters part of the solvent, in which they freely float, into their interior [83]. Their advantage, with respect to pharmaceutical application as drug carriers, is the wide variety of drugs to be incorporated as well as the biocompatibility, inherently connected with natural phospholipids. Regarding the penetration behavior of liposomes it is still under discussion if such objects might penetrate intact skin [84-87].

Liposomes have become one of the pharmaceutical nanocarriers of choice for many applications. Currently, many liposome-based drugs and biomedical products have been approved for use in clinic. They were used to study membrane processes and membrane-bound proteins. Liposomes were also proposed as drug carriers that reduce toxicity and increase efficacy. The nature of liposomes makes them one of the best alternatives for drug delivery because they are nontoxic and remain inside the bloodstream for a long time. They are being successfully used in cancer therapy and in skin melanoma [6]. However, to date many liquid-type nanocosmetic carriers, such as liposomes, are structurally unstable. Specifically, when passing through the skin, they adhere to the inside walls of the skin cells, causing the collapse of

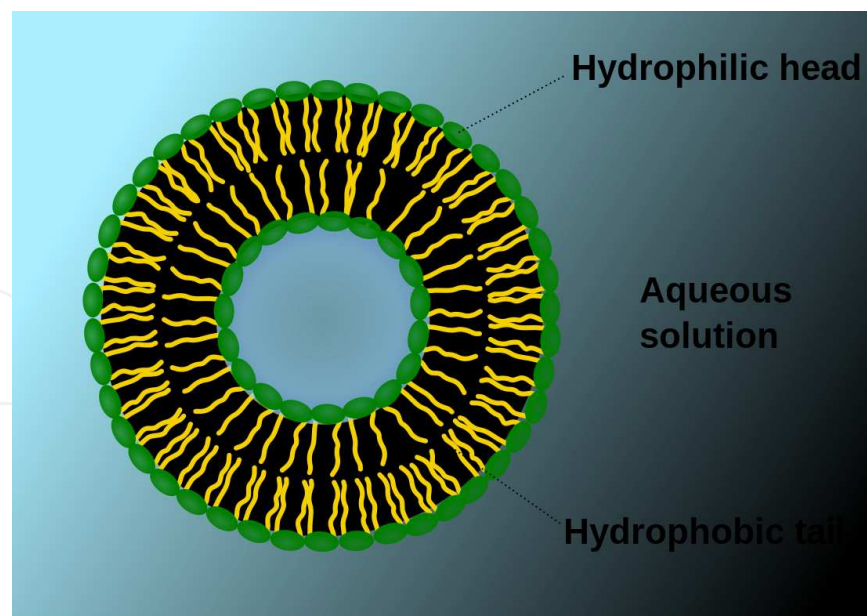


Figure 5. Structure of a liposome (<http://en.wikipedia.org/wiki/Liposome>. Downloaded on April 26, 2014)

phospholipid-association bodies and the leak of their encapsulated ingredients. As a result, their ability to transport active ingredients to deep skin is not likely good. For this reason, the use of flexible liposomes (transformable liposomes or transfersomes) has emerged as an invaluable strategy to reach the objective of drug delivery via the transdermal route. Examples of drugs delivered throughout the skin by using liposomes are melatonin, indinavir, methotrexate, amphotericin B, ketoprofen, estradiol, clindamycin hydrochloride, and lignocaine, while examples of transdermal drug delivery using transformable liposomes (transfersomes) are diclofenac, insulin, tetanus toxoid, corticosteroids, superoxide dismutase, DNA, triamcinolone-acetonide, ketoprofen, interleukin-2, and ketotifen fumarate [88-94].

10.3.2. Niosomes

These are non-ionic surfactant vesicular novel drug delivery system in which the medication is encapsulated in a vesicle. Niosomes are unilamellar or multilamellar vesicles capable of entrapping hydrophilic and hydrophobic solutes. From a technical point of view, niosomes are promising drug carriers as they possess greater stability and lack of many disadvantages associated with liposomes, such as high cost and the variable purity problems of phospholipids [95]. Another advantage is the simple method for the routine and large scale production of niosomes without the use of unacceptable solvents. One alternative of phospholipids is the hydrated mixture of cholesterol and non-ionic surfactants such as alkyl ethers, alkyl esters or alkyl amides [95,96]. This type of vesicle formed from the above mixture has been known as niosomes or non-ionic surfactant vesicles. Niosome surfactants are biodegradable, biocompatible and non-immunogenic.

Niosomes are versatile carrier systems that can be administered through various routes, including transdermal delivery [97,98]. Particular efforts have been aimed at using niosomes

as effective dermal and transdermal drug-delivery systems [99,100]. In particular, niosomes are considered an interesting drug-delivery system in the treatment of dermatological disorders. Examples of transdermal drug delivery using niosomes are minoxidil and ellagic acid. Niosomes have been reported to enhance the residence time of drugs in the stratum corneum and epidermis, while reducing the systemic absorption of the drug, and improve penetration of the trapped substances across the skin. In addition, these systems have been reported to decrease side effects and to give a considerable drug release [101]. Niosomes formed from sorbitan monoesters (Spans) with cholesterol molar ratios of 1:1 are a promising approach for the topical delivery of minoxidil in hair-loss treatment [102]. Junyaprasert et al demonstrated that the Span 60 and Tween 60 niosomes may be a potential carrier for dermal delivery of ellagic acid [103].

10.3.3. *Transfersomes*

Transfersomes have been defined as specially designed vesicular particles, consisting of at least one inner aqueous compartment surrounded by a lipid bilayer with appropriately tailored properties. Accordingly, transfersomes resemble lipid vesicles, liposomes, in morphology but, functionally, transfersomes are sufficiently deformable to penetrate pores much smaller than their own size [104].

They are metastable, which makes the vesicle membrane ultraflexible, and, thus, the vesicles are highly deformable. It is chiefly the unusually strong membrane adaptability that allows the transfersomes vesicles to accommodate to a confining pore and thus trespass such a pore. Typical transfersomes are, therefore, characterized by at least one order of magnitude more elastic membrane than that of conventional lipid vesicles, liposomes. In order to change liposomes into transfersomes, one can incorporate one or more edge-active substance(s) into the vesicular membrane, surfactants were suggested as examples of such edge-activators [105-107]. Another specific difference between transfersomes and liposomes is the higher hydrophilicity of the former, which allows transfersome membrane to swell more than conventional lipid vesicle bilayers.

10.3.4. *Ethosomes*

Ethosomes are lipid vesicular carriers embodying ethanol in relatively high concentrations for enhanced skin permeation of drugs [108]. They are composed mainly of phospholipids, ethanol and water. The high concentration of ethanol, which essentially differentiates ethosomes from other vesicular carriers, acts to enhance skin permeation in order to release the entrapped drug particles into deeper layers and systemic circulation. Ethosome was developed by Touitou in 1996, in the course of studying the use of lipid vesicles in drug delivery systems for skin treatment [109].

Structurally, an ethosomal vesicle is composed a phospholipid bilayer and an aqueous inner core containing the entrapped active ingredient. They are soft and malleable. The size of an ethosome vesicle lies within the nanometer range [110]. In addition, the size of ethosome vesicle is smaller than that of a liposome when prepared under the same condi-

tions, due to the high alcohol content. The size decreased as alcohol increased from 20 to 45 % [39]. This reduction in size was attributed to the conferment of a net negative charge on the vesicle surface by ethanol. Other excipients usually added in ethosome formulation include cholesterol, for vesicle membrane stabilization; permeation enhancers, marker dyes (if required) such as rhodamine, for characterization study. The stabilizing effect of cholesterol is due to prevention of vesicle aggregation and enlargement during storage [111]. The small size and malleability of ethosomes enable them to pass through the skin or membrane barrier and also influence the extent of transdermal permeation. The smaller the size, the greater the extent of penetration [108].

Ethosomes permeate through the stratum corneum barrier and possess significantly high transdermal flux unlike classical liposomes. These effects of combined phospholipids and high concentration of ethanol in vesicular formulations have been suggested to be responsible for deeper distribution and penetration in the skin lipid bilayers [110]. Application of ethosomes in drug delivery has numerous advantages [112-114]: simplicity of the technology, non-invasive means of application (e.g., topical), enhanced transdermal drug delivery, and avoidance of first-pass effect. Non-invasiveness enhances patient's compliance hence, better therapeutic outcome. Ethosomes have been shown to exhibit high encapsulation efficiency for a wide range of molecules including lipophilic drugs due to the multilamellarity of the vesicles as well as the presence of ethanol, which allows for better solubility of many drugs [39,115]. Unlike liposomes and transfersomes, ethosomes were able to improve skin delivery of drugs both under occlusive [117] and non-occlusive conditions [111,118,119].

The application of transformable liposomes, which are prepared using alcohol (ethosomes) in the lipid bilayer of stratum corneum, able to deform and penetrate throughout the skin when pressure is applied, has been increased. For example, tacrolimus-loaded ethosomes may be useful as a therapeutic agent for atopic dermatitis [120]. Skin permeation of ethosomal formulations assessed by confocal microscopy revealed enhanced permeation of Rhodamine 123-loaded formulation in comparison to the hydroalcoholic solution. Another ethosomal formulation has proved to be a potentially useful vehicle for transdermal delivery of ketoprofen [121]. Furthermore, an ethosomal carrier (phosphatidylethanolamine) is an optional treatment for psoriasis that provides long-term therapeutic effects, is nontoxic, and has better compliance with patients. Application of ethosomal carriers with 5-aminolevulinic acid (ALA) in hyperproliferative murine skin can improve the penetration of ALA and the formation of protoporphyrin IX and significantly reduce tumor necrosis factor in this disordered skin compared to an ALA aqueous solution [122].

Ethosomes were used efficiently to enhance the anti-inflammatory activity of ammonium glycyrrhizinate compared to the ethanolic or aqueous solutions of this drug [123]. Moreover, the ethosomal system dramatically enhanced the skin permeation of minoxidil *in vitro* compared with either ethanolic or hydroethanolic solution or phospholipid ethanolic micellar solution of minoxidil. In addition, the transdermal delivery of testosterone from an ethosomal patch was greater both *in vitro* and *in vivo* than from commercially available patches [124]. Examples of transdermal drug delivery using ethosomes are tacrolimus [120], clotrimazole [125], trihexyphenidyl HCl [126], ketoprofen [121] and testosterone.

10.4. Dendrimers

Dendrimers are nonpeptidic fractal 3-D structures made of numerous small molecules. The structure of these molecules results in relatively uniform shapes, sizes, and molecular weights. The permeability of dendrimers through the skin depends on physicochemical characteristics like generation size, molecular weight, surface charge, composition, and concentration. These nanocarriers have been used to transport photosensitizers for photochemical therapy and antifungal molecules.

Dendrimers have been utilized for transdermal drug delivery, as shown in Table 1. The main problems with this kind of transdermal carrier are their poor biodegradation and inherent cytotoxicity [127]. The main advantage of dendrimers is that they have multivalency [128] and it is possible to precisely control the functional groups on the surface [129]. Due to their form and size, these molecules can carry drugs, imaging agents, etc. Dendrimers interact with lipids present in membranes, and they show better permeation in cell cultures and intestinal membranes. Dendrimers also act like solubility enhancers, increasing the permeation of lipophilic drugs. However, they are not good carriers for hydrophilic drugs. Examples of drugs delivered throughout the skin by using dendrimers are tamsulosin [130], indomethacin [131], ketoprofen, diflunisal [132], 5-fluorouracil [133] and peptides [134].

Nanocarrier	Advantages	Disadvantages
Nanoparticles	<ul style="list-style-type: none"> • They can be made of a lot of biodegradable materials. • There are many ways to prepare them. • They can include antibodies in their surface to reach target organs. • Both hydrophilic and hydrophobic drugs can be loaded in ananoparticle. • They are able to avoid the immune system due to their size. 	<ul style="list-style-type: none"> • Not enough toxicological assessment has been done. • It is difficult to develop an analytical method for drug delivery. • Some processes are difficult to scale up. • Sometimes, the size they reach is not enough to avoid the immune system.
Nanoemulsions	<ul style="list-style-type: none"> • They can be formulated as foams, liquids, creams, and sprays. • They are nontoxic and nonirritant. • Easily applied to skin and mucous membranes. 	<ul style="list-style-type: none"> • They are susceptible to Oswald ripening. • Surface charge has a marked effect on stability. • Variable kinetics of distribution processes and clearance.
Liposomes	<ul style="list-style-type: none"> • Control release based on natural lipids. 	<ul style="list-style-type: none"> • When high-pressure homogenization is used, decreased stability of high molecular weight molecules.

Nanocarrier	Advantages	Disadvantages
Dendrimers	<ul style="list-style-type: none"> • High biocompatibility. 	<ul style="list-style-type: none"> • Lipid crystallization leads to a lot of polymorphic issues.
	<ul style="list-style-type: none"> • Simple manufacture. 	<ul style="list-style-type: none"> • Variable kinetics of distribution processes.
	<ul style="list-style-type: none"> • Protein carriers increase their stability. 	<ul style="list-style-type: none"> • They are susceptible to physical instability.
	<ul style="list-style-type: none"> • High drug loads. 	
	<ul style="list-style-type: none"> • They increase stability of therapeutic agents. • They are easily prepared and functionalized. 	<ul style="list-style-type: none"> • They have shown cellular toxicity. • Elimination and metabolism could be a problem depending on the generation and materials.
	<ul style="list-style-type: none"> • They increase bioavailability of drugs. 	<ul style="list-style-type: none"> • Their synthesis costs are higher than other nanocarriers.
Niosomes, transfersomes, ethosomes	<ul style="list-style-type: none"> • Biodegradable and low toxicity. 	<ul style="list-style-type: none"> • Predisposition to oxidative degradation.
	<ul style="list-style-type: none"> • Easy to prepare. 	<ul style="list-style-type: none"> • Purity of natural phospholipids.
	<ul style="list-style-type: none"> • Softness, malleability. 	<ul style="list-style-type: none"> • Formulations may be expensive.
	<ul style="list-style-type: none"> • They can encapsulate both hydrophilic and lipophilic moieties. 	
	<ul style="list-style-type: none"> • Ability to target organs for drug delivery. 	
	<ul style="list-style-type: none"> • Extremely high flexibility of their membrane. 	

Table 2. Advantages and disadvantages of nanocarrier systems for transdermal drug delivery [6, 75].

10.5. Lipid nanoparticles

Lipid nanoparticles include solid lipid nanoparticles (SLN), nanostructured lipid carriers, lipid drug conjugates and are colloidal drug carrier systems [135-137]. They are very much like nanoemulsions, differing in lipid nature. The liquid lipid used in emulsions is replaced by a lipid solid at room temperature in SLN including high-melting point glycerides or waxes [136,138,139]. Lipid nanoparticles are good candidates for transdermal delivery. They can be prepared in different sizes and it is possible to modify surface polarity in order to improve skin penetration. From the upper skin, nanoparticles can reach deeper skin regions because they exhibit mechanical flexion [6].

10.5.1. Nanostructured lipid carriers (NLC)

NLC are colloidal carriers characterized by a solid lipid core consisting of a mixture of solid and liquid lipids, and having a mean particle size in the nanometer range. They consist of a

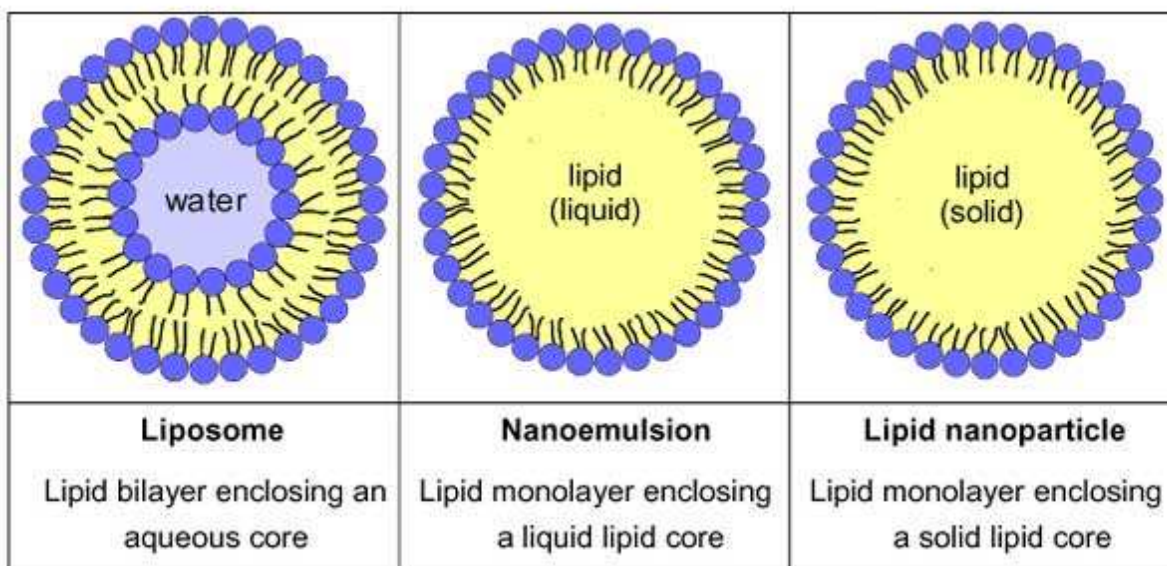
lipid matrix with a special nanostructure [140]. This nanostructure improves drug loading and firmly retains the drug during storage. NLC system minimizes some problems associated with SLN such as low payload for some drugs; drug expulsion on storage and high water content of SLN dispersions.

The conventional method for the production of NLC involves mixing of spatially very different lipid molecules, i.e. blending solid lipids with liquid lipids (oils). The resulting matrix of the lipid particles shows a melting point depression compared with the original solid lipid but the matrix is still solid at body temperature. Depending on the method of production and the composition of the lipid blend, different types of NLC are obtained. The basic idea is that by giving the lipid matrix a certain nanostructure, the payload for active compounds is increased and expulsion of the compound during storage is avoided. Ability to trigger and even control drug release should be considered while mixing lipids to produce NLC. Newer methods of generating NLC have been developed [141].

10.5.2. Solid lipid nanoparticles

Solid lipid nanoparticles (SLN) are formed by a matrix of lipids which are biodegradable raw materials that are physiologically well tolerated [142]. The main advantages of these systems include protection of labile substances from chemical degradation, control of the release of substances due to the solid state of the lipid matrix, and formation of films over the skin showing occlusive properties [140]. Additional features are the avoidance of organic solvents during the preparation and amenability to large scale production and sterilization. Furthermore, the great ability of SLNs to facilitate the contact of active substances with the stratum corneum, because of the small size of the particles and consequently the high surface area, leads to the high permeation of the carried substances through the viable skin [143].

The degree of crystallinity of lipid nanoparticles has a great impact on the extent of occlusion by the formulation. With increasing crystallinity, the occlusion factor increases as well [142]. This explains why liquid nanoemulsions in contrast to SLNs do not show an occlusive effect and why the extent of occlusion by nanostructured lipid carrier (NLC) compared to SLN is reduced. Other parameters influencing the occlusion factor are the particle size and the number of particles. The occlusion factor decreases with increase in particle size but increases with increase in number of particles. The occlusive effect leads to reduced water loss and increased skin hydration. Highly crystalline SLNs can be used for physical sun protection due to scattering and reflection of the ultra violet (UV) radiation by the particles. A high crystallinity was found to enhance the effectiveness and was also synergistic with UV absorbing substances used in conventional sunscreens. Similarly, synergism was observed on the sun protection factor and UV-A protection factor exhibited by the incorporation of the inorganic sunscreen, titanium-dioxide in NLC of carnauba wax and decyloleate [144]. Fig. 6 shows the structures of some lipid nanodispersed vehicle systems.



(<http://www.skin-care-forum.basf.com/en/author-articles/strategies-for-skin-penetration-enhancement/2004/08/12?id=5b9a9164-6148-4d66-bd84-6df76bd6d111&mode=Detail>. Downloaded April 26, 2014).

Figure 6. Structure of lipid nanodispersed vehicle systems

10.6. Polymeric nanoparticles

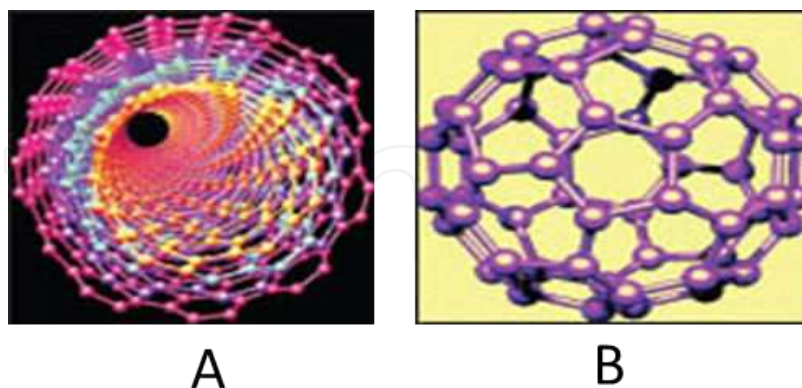
Polymeric nanoparticles are prepared from biocompatible and biodegradable polymers with size between 10-1000 nm, where the drug is dissolved, entrapped, encapsulated or attached to a polymer nanoparticle matrix. The penetration and transport extent of these systems through the skin depends on the ingredients' chemical composition, the encapsulation mechanism influencing the drug release, the size of nanoparticles and on the viscosity of the formulations. The polymeric nanoparticles are able to modify the activity of drugs, delay and control the drug release, and increase the drug adhesivity or its time of permanence in the skin. Briefly, the nanoparticles can be useful as reservoirs of lipophilic drugs to deliver them in the stratum corneum becoming an important strategy to control their permeation into the skin [145].

10.7. Carbon nanotubes (CNT) and fullerenes

Carbon nanotubes are stable carbon nanoparticles with potential anti-oxidant ability and cytoprotective effect. Carbon nanotubes (Fig. 7) have extremely small mean diameters (<100 nm). Their large inner volume allows the loading of small biomolecules while their outer surface can be chemically modified to render themselves various novel features that can be used to load proteins and genes for effective drug delivery [146], even through the skin.

Fullerenes (Fig. 7) are 1-nm scale carbon spheres of 60 carbon atoms. Although fullerenes are hydrophobic, they can be organically functionalized by attaching hydrophilic moiety and become water-soluble and capable of carrying genes, proteins and other biomolecules for delivery purposes [146]. Their small size, spherical shape and hollow interior all provide

therapeutic opportunities and have been proposed for use in cosmetic products like sunscreens, moisturizers, long lasting makeup, etc.



<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3853888/figure/F4/#108;93> A; <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3853888/figure/F5/#117.75;90.75> B

Figure 7. Carbon nanotube (A) and fullerene (B)

11. Influence of nanoparticulate formulations on biochemical processes of the skin

Apart from environmental protection against radiation, functions of the skin include heat regulation, immune response, biochemical synthesis, sensory detection, regulation of absorption/loss of water and electrolytes. The stratum corneum formed from nonviable corneocytes plays the major role.

A crucial question for the investigation of nanoparticulate drug delivery carriers is the site of drug release from the particles, i.e., does the release occur in suspension or on the skin surface leaving the carrier particles outside or do the particles penetrate the skin to release the drug within the tissue? Nanoparticles formulations for dermal delivery or transdermal delivery influenced some of the traditional functions of the skin. Once applied to the skin, enzymes activated by body heat led to the formation of an active ingredient (allyl isothiocyanate). Transport of the active drug component took place by passive diffusion across the skin-the very basis of transdermal drug delivery [147,148]. The alcohol in ethosomes initiates the process of transdermal permeation and drug release by its permeation enhancing effect [149]. The major hindrance to TDDS is the stratum corneum layer that forms a strong barrier and limiting factor to skin penetration and permeation of many drugs.

The processes involved in drug delivery from ethosomes through the skin are illustrated in Fig. 8 [46]. The alcohol makes the vesicles to be packed loosely and the vesicle membranes to become softer and malleable [13]. It also causes reversible perturbations in the deeper layers of SC and penetrates intercellular lipid layers of skin cell membranes making them more fluid

and less dense [150]. The vesicles then squeeze through the intercellular spaces into the deeper layers of skin. It has been shown that drug particles are concentrated more on the inside wall than in the core of vesicles [151]. In this position, release of the vesicular content is thermodynamically favored. Owing to the increased affinity, due to its lipid content, the vesicle fuses with the lipid contents of the skin layers and releases its content which then diffuses into deeper layers of the skin or membrane and into systemic circulation. Other mechanisms, such as the free drug diffusion, may be involved in penetration.

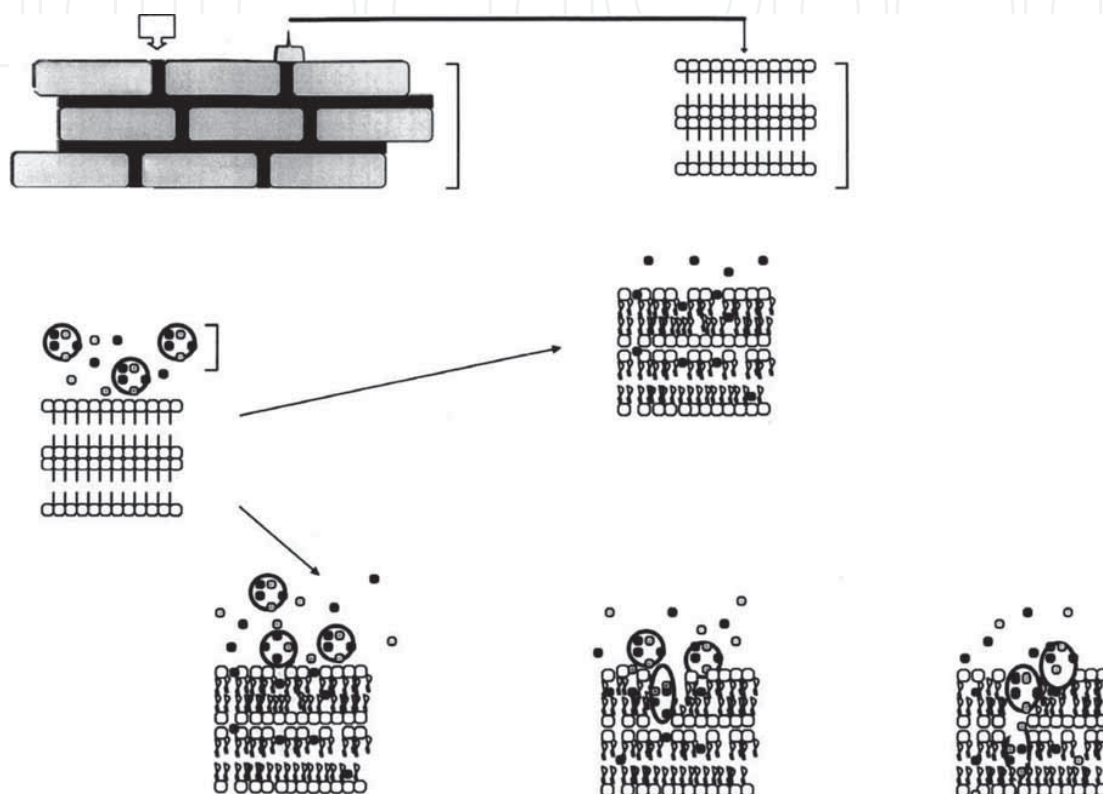


Figure 8. Mechanism of drug delivery from ethosomal vesicular carriers through the skin [46,115]. Note the initial fluidization of the skin architecture.

12. Regulations on dermal and transdermal delivery systems

Safety and toxicological issues are the most important issues for a drug delivery system. Safety is an obvious concern for the fast growth of nanoparticles mediated drug delivery [152]. Governmental regulatory agencies such as the United States Food and Drug Agency (USFDA) have established guidelines describing the kind of safety tests that should be conducted in animals in order to have a new drug approved for use in clinical trials and in order to get approval of a new drug application (NDA) for marketing. The rationale and circumstances for conducting reproductive, mutagenicity, carcinogenicity, irritation, and sensitization studies have already been mentioned. The requirements for acute, subacute, and chronic toxicity

studies for pharmaceutical products intended for use in humans as described according to are the requirements of the United States, Japan, and Europe because these areas represent the largest pharmaceutical markets in the world today. These requirements have been developed at the International Conference on Harmonization to provide uniformity among the three regions [153]. Phases I, II, and III refer to the different phases of human clinical trials. Phase I refers to the initial trials, limited to one or a few doses to determine absorption, pharmacokinetics, and an initial estimate of safety. Phase II refers to larger scale studies to establish safety and to get an initial estimate of clinical efficacy. Phase III refers to the final, large-scale, multicenter trials aimed at establishing efficacy.

The Food and drug agency (FDA) paradigm for regulation of new products is based on the concepts of risk management, which includes identification, analysis and control of risk [154]. The regulation and approval by the FDA is on a “product by product” basis, with the overall regulation process falling into three stages: premarket approval, premarket acceptance and post-market surveillance.

Premarket approval: Prior to market introduction of any new pharmaceuticals, high-risk medical devices, food additives, colors, and biologicals, FDA approval is required. The producer/sponsor of the product is responsible for identifying and assessing the risks presented by the product. This party will also be responsible for indicating means to minimize the risks in a product application.

Premarket acceptance: This category refers to products that are often copies of similar products that were approved previously or are products prepared according to approved specifications. For these products, the FDA receives and reviews some form of notice that the products will be marketed and the products undergo a more rapid review process than premarket approval.

Postmarket surveillance: In this category, FDA manages the risks of GRAS products like foods, cosmetics, radiation emitting electronic products and materials such as food additives and food packaging. For products in this category, market entry, and distribution are at the discretion of the manufacturer/producer. These products are generally regulated by the application of good manufacturing practices. FDA takes regulatory action if adverse events that threaten public or individual health occur.

The FDA coordinates policies within itself and with other government agencies. As and when new toxicological risks that derive from the new materials and/or new conformations of existing materials are identified, the FDA will require new tests.

The FDA regulations are for products, not technologies. In addition, the FDA regulates only the claims made by the product sponsor. If the manufacturer makes no nanotechnology claims regarding the manufacture or performance of the product, the FDA may be unaware at the time that the product under review employed nanotechnology. Finally, the FDA has only limited authority over some potentially high-risk products, such as cosmetics. Many products are regulated only if they cause adverse health-related events in use. To date there have been few resources available to assess the risks of these products.

13. Dermal and transdermal formulations on the market

A lot of dermal and transdermal drug delivery systems have been licensed for manufacture after passing through the regulatory approval and trials as specified by different countries example FDA (United States of America). Some of the drugs currently available on the market are presented in Table 3.

Drug	Trade name	Type of transdermal patch	Manufacturer	Indication
Fentanyl	Duragesic	Reservoir	Alza/Janssen Pharmaceutica	Moderate/ Severe pain
Nitroglycerine	Deponit	Drug in adhesive	Schwarz Pharma	Angina Pectoris
	Minitran	Drug in adhesive	3M Pharmaceuticals	
	Nitrodisc	Micro reservoir	Searle, USA	
	Nitrodur	Matrix	Key Pharmaceuticals	
	TransdermNitro	Reservoir	Alza/Novartis	
	Nitroderm TTS	Face	Novartis	
	Diafusor	Matrix	Schering-Plough	
	Transdermal-NTG	Rim	Warner Chilcott Lab	
	Nitrocine	Rim	Kremer Urban	
	Nitro patch	Rim	Adria Lab	
	NTS patch	Rim	Bolar, Major, Qualitest, Bio-Line, Goldline, Geneva, Rugby WarnerChilcott Lab	
Isosorbide dinitrate	Frاندول Tape	Matrix	Toaeiyo, Yamanouchi Pharm.	
Nicotine	Prostep	Reservoir	ElanCorp/Lederie Labs	Smoking Cessation
	Nicotrol	Drug in adhesive	Cygnus Inc./McNeil Consumer Products Ltd.	
	Nicotinell	Matrix	Novartis	
	Nikofrenon	Matrix	Novartis	
	Habitraol	Drug in adhesive	Novartis	
Testosterone	Androderm	Reservoir	Thera Tech/ GlaxoSmithKline	Hypogonadism in males
	Testoderm TTS	Reservoir	Alza	
Clonidine	Catapres-TTS	Membrane matrix hybrid type	Alza/Boehinger Ingelheim	Hypertension
Lidocaine	Lidoderm	Drug in adhesive	Cerner Multum, Inc.	Anesthetic

Drug	Trade name	Type of transdermal patch	Manufacturer	Indication
Scopolamine	Transderm Scop	Membrane matrix hybrid type	Alza/Novartis	Motion sickness
Hyoscine	Trasiderm-Scop	Matrix	Novartis	
	Kimite-patch	Matrix	Myun Moon Pharm. Co.	
Minoxidil 4%	Nanominox		Sinere, Germany	Hair growth promoter
Acyclovir	Supravir cream		Trima, Israel	herpes infection.
Many ingredients	Cellutight EF		Hampden Health, USA	Topical cellulite
Estradiol	Climara	Drug in adhesive	3M Pharmaceuticals/ Berlex Labs	Postmenstrual Syndrome
			Noven Pharma/Novartis	
	Vivelle	Drug in adhesive	Alza/Novartis.	
	Estraderm	Reservoir	Women First Healthcare, Inc	
	Esclim	Drug in adhesive	Johnson & Johnson	
	Ethinyl Estradiol	Ortho Evra	Drug in adhesive	

Table 3. Currently available medications for transdermal delivery [155,156].

14. Dermal and transdermal delivery of phytopharmaceuticals

Novel drug delivery system is a novel approach to drug delivery that addresses the limitations of the traditional drug delivery systems. Phytopharmaceuticals are pharmaceuticals using traditional compounds derived from botanicals instead of chemicals. Because these natural ingredients are more easily and more readily metabolized by the body they produce fewer if any side effects and provide increased absorption in the bloodstream resulting in more thorough and effective treatments unlike pharmaceuticals produced from chemical compounds which are prone to adverse side effects [157]. The formulation of dermal and transdermal delivery of phytopharmaceuticals is gaining interest owing to the benefits accruable from it. One of the first few attempts to utilize TDDS containing phytopharmaceuticals was investigation aimed to formulate transdermal films incorporating herbal drug components such as boswellic acid (*Boswellia serrata*) and curcumin (*Curcuma longa*), which utilizes skin as a site for continuous drug administration into the systemic circulation [157]. TDDS avoids first pass metabolism of the drug without the pain associated with injection; moreover the system provides a sustained drug delivery with infrequent dosing via zero-order kinetics and the therapy can easily be terminated at any time. For the local action of the drug at the site of administration of TDDS, turmeric are used which is considered a new version of ayurvedic turmeric poultice or *lepa* [158].

Application of vesicular encapsulation holds great promise in the development and use of phytomedicines considering the difficulties of their formulation into stable dosage forms. Certain physicochemical properties of many herbal extracts make their formulation difficult due to stability and processing challenges. By using appropriate techniques, vesicular products of herbal extracts with enhanced stability and efficacy have been produced. A new drug delivery device known as phytosome, composed of phosphatidylcholine, has been developed to overcome the poor absorption of flavonoids, a challenge due mainly to their large molecular sizes and poor miscibility with the lipid contents of cell membrane linings [159]. Phytosomes are well absorbed when taken orally.

Evaluations of phytosomes indicate that a bond is formed between a flavonoid and a phosphatidylcholine molecule to form a hybrid that is highly lipid-miscible. The development and applications of a variety of novel vesicular herbal formulations such as liposomes, phytosomes, transfersomes and ethosomes have been reported [160,161]. Ethosomes, by virtue of their special characteristics, may circumvent the hindrances to successful delivery of phytomedicines. Both soluble and insoluble phytomedicines can be encapsulated in ethosomes. Ethosomes also offer protection from premature degradation and increased biodistribution, which would make for improved bioavailability and more beneficial therapeutic outcome for TDDS.

15. Conclusion

From the myriad published studies involving nanoparticles, it is clear that nanoparticles have the potential to effectively deliver drugs across the skin barrier. Conventional liposomes, flexible liposomes, ethosomes, niosomes and ultradeformable liposomes, etc offer potential value as dermal and transdermal drug delivery systems in addition to other lipid nanoparticles.

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