Chapter 8

Nanocarrier Systems for Transdermal Drug Delivery

José Juan Escobar-Chávez, Isabel Marlen Rodríguez-Cruz, Clara Luisa Domínguez-Delgado, Roberto Díaz-Torres, Alma Luisa Revilla-Vázquez, Norma Casas Aléncaster

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/50314

1. Introduction

The nanomedicine which is the application of technologies on the scale of 1 to 500 nm to diagnose and treat diseases, it has become a very relevant topic nowadays. During the last century, there has been a lot of new research and patents regarding nanomedicine in health sciences [1]. The objective of nanomedicine is to diagnose and preserve the health without side effects with noninvasive treatments. To reach these goals, nanomedicine offers a lot of new tools and capabilities. The manipulation that nanomedicine provides to the drugs and other materials in the nanometer scale can change the basic properties and bioactivity of materials. The solubility, increment in surface area, control release and site-targeted delivery are some characteristics that nanotechnology can manipulate on drug delivery systems.

Nanotechnology applied to health sciences contains new devices used in surgery, new chips for better diagnostics, new materials for substituting body structures and some structures capable to carry drugs through the body for treatment of a lot of diseases. These structures can be made of a lot of different materials and they are very different in structure and chemical nature. All these nanostructures are called nanocarriers and they can be administrated into the organisms by topical and transdermal routes [2]. Nanocarriers are a powerful weapon against a lot of illnesses since they are so small to be detected by immune system and they can deliver the drug in the target organ. For that reason, drug doses using nanocarriers and side effects decrease a lot.

The idea for using these tiny systems is not as new as we think but the use of nanocarriers in pharmaceutical products is not frequent, since the technology is expensive for certain types of nanoparticles and because nanocarriers need to be evaluated for demonstrating they do not have toxic effects. Nowadays the controversy of biological effects due to nanostructures

is an open discussion, in one hand, the nanotechnologist continue making new and more sophisticated nanocarriers and in the other hand, toxicologist continue evaluating possible damaging effects.

Whatever it happens, nanotechnology is the new era and nanomedicine cannot be taking off. New nanocarriers will be created and the entire scientist working in nanomedicine bet for it to be the cure of diseases that in this moment are difficult to deal with [3]

The application of preparations to the skin for medical purposes is as old as the history of medicine itself, with references to the use of ointments and salves found in the records of Babylonian and Egyptian medicine. The historical development of permeation research is well described by Hadgraft & Lane [4]. Over time, the skin has become an important route for drug delivery in which 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. In order to enhance drug transdermal absorption different methodologies have been investigated developed and patented [5,6]. 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 [3,7-10].

A number of excellent reviews that have been published contain detailed discussions concerning many aspects of transdermal nanocarriers [11-17]. The present chapter shows an updated overview of the use of submicron particles and other nanostructures in the pharmaceutical field, specifically in the area of topical and transdermal drugs. This focus is justified due to the magnitude of the experimental data available with the use of these nanocarriers. The development of submicron particles and other nanostructures in the pharmaceutical and cosmetic fields has been emerged in the last decades for designing best formulations for application through the skin [18-21].

2. The skin

The skin is the largest organ of the body [22-24], accounting for more than 10% of body mass, and the one that enables the body to interact more intimately with its environment. Essentially, the skin consists of four layers: The SC, that is the outer layer of the skin (non-viable epidermis), and forms the rate-controlling barrier for diffusion for almost all compounds. It is composed of dead flattened, keratin-rich cells, the corneocytes. These dense cells are surrounded by a complex mixture of intercellular lipids, namely, ceramides, free fatty acids, cholesterol, and cholesterol sulphate. Their most important feature is that they are structured as ordered bilayer arrays [25-28]. The other layers are: the remaining layers of the epidermis (viable epidermis), the dermis, and the subcutaneous tissue (**Figure 1**). There are also several associated appendages: hair follicles sweat ducts, glands and nails [29,30].

Many agents are applied to the skin either deliberately or accidentally, with either beneficial or deleterious outcomes. The main interest in dermal absorption assessment is related to: a) Local effects in dermatology (e.g., corticosteroids for dermatitis); b) transport through the skin seeking a systemic effect (e.g., nicotine patches, hormonal drug patches, etc.) [31]; c) surface effects (e.g., sunscreens, cosmetics, and anti-infectives) [32,33]; d) targeting of deeper tissues (e.g., nonsteroidal anti-inflammatory agents) [34-37]; and e) unwanted absorption (e.g., solvents in the workplace, pesticides or allergens) [38,39].

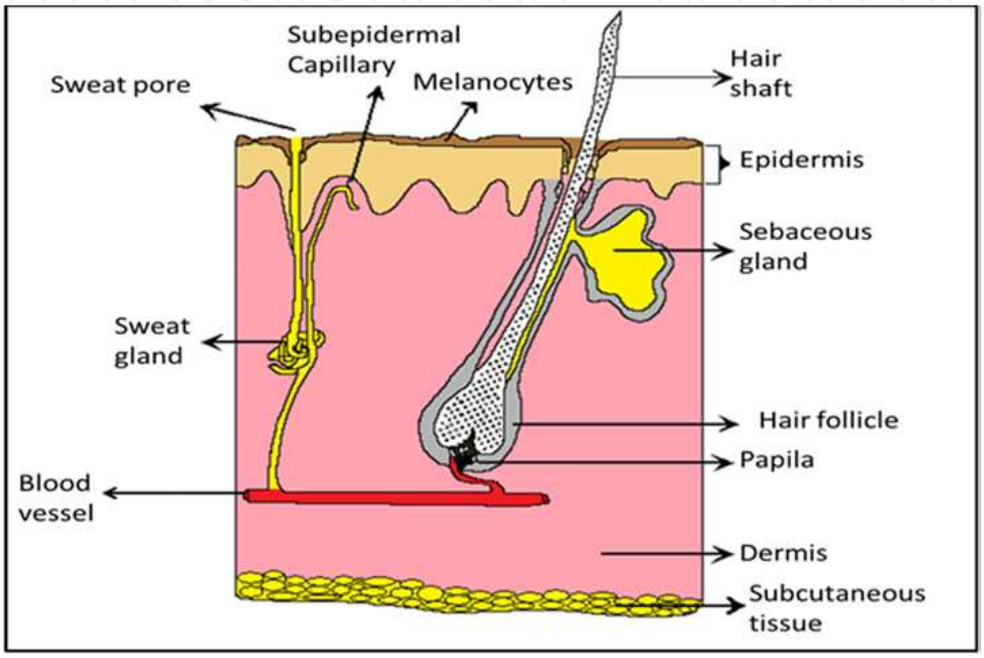

Figure 1. Schematic representation of skin layer.

2.1. Epidermis

2.1.1. Stratum corneum

The stratum corneum is the heterogeneous outermost layer of the epidermis and is approximately 10-20 μm thick. The stratum corneum consists of about 15 to 25 layers of flattened, stacked, hexagonal, and cornified cells embedded in an intercellular matrix of lipids. These lipid domains form a continuous structure so they are considered to play a crucial role in the maintenance of the skin barrier that helps avoid transepidermal water loss. Each cell is approximately 40 μm in diameter and 0.5 μm thick [40].

The stratum corneum barrier properties may be partly related to its very high density (1.4 g/cm^3 in the dry state) and its low hydration of 15–20 %, compared with the usual 70 % for the body. Each stratum corneum cell is composed mainly of insoluble bundled keratins (70 %) and lipid (20 %) encased in a cell envelope, accounting for about 5% of the stratum corneum weight. The permeability barrier is located within the lipid bilayers in the intercellular spaces of the stratum corneum [6-8] and consists of ceramides (40–50%), fatty acids (15–25%), cholesterol (20–25%) and cholesterol sulphate (5–10 %) [41-45].

The barrier function is further facilitated by the continuous desquamation of this horny layer with a total turnover of the stratum corneum occurring once every 2–3 weeks. The stratum corneum functions as a barrier are to prevent the loss of internal body components, particularly water, to the external environment. The cells of the stratum corneum originate in the viable epidermis and undergo many morphological changes before desquamation. Thus, the epidermis consists of several cell strata at varying levels of differentiation.

The origins of the cells of the epidermis lie in the basal lamina between the dermis and viable epidermis. In this layer there are melanocytes, Langerhans cells, Merkel cells, and two major keratinic cell types: the first functioning as stem cells having the capacity to divide and produce new cells; the second serving to anchor the epidermis to the basement membrane [46]. The basement membrane is 50–70 nm thick and consists of two layers, the lamina densa and lamina lucida, which comprise mainly proteins, such as type IV collagen, aminin, nidogen and fibronectin. Type IV collagen is responsible for the mechanical stability of the basement membrane, whereas laminin and fibronectin are involved with the attachment between the basement membrane and the basal keratinocytes. The cells of the basal lamina are attached to the basement membrane by hemidesmosomes, which are found on the ventral surface of basal keratinocytes [47]. Hemidesmosomes appear to comprise three distinct protein groups: two of which are bullous pemphigoid antigens (BPAG1 and BPAG2), and the other epithelial cellspecific integrins [48-50]. BPAG1 is associated with the organization of the cytoskeletal structure and forms a link between the hemidesmosome structure and the keratin intermediate filaments. The integrins are transmembrane receptors that mediate attachment between the cell and the extracellular matrix. Human epidermal basal cells contain integrins $\alpha_2\beta_1$, $\alpha_3\beta_1$ and $\alpha_6\beta_4$. Integrin $\alpha_6\beta_4$ and BPAG2 appear to be the major hemidesmosomal protein contributors to the anchoring of the keratinocyte, spanning from the keratin intermediate filament, through the lamina lucida, to the lamina densa of the basement membrane [51]. In the lamina densa, these membrane-spanning proteins interact with the protein laminin-5 which, in turn, is linked to collagen VII, the major constituent of the anchoring fibrils within the dermal matrix. It has also been suggested that both BPAG2 and integrin $\alpha_6\beta_4$ mediate in the signal transductions required for hemidesmosome formation and cell differentiation and proliferation. Integrin $\alpha_3\beta_1$ is associated with actin and may be linked with laminin-5. Epidermal wounding results in an up-regulation of these proteins that appears to be involved with cell motility and spreading. The importance of maintaining a secure link between the basal lamina cells and the basement membrane is obvious, and the absence of this connection results in chronic blistering diseases such as pemphigus and epidermolysis bullosa.

2.2. Dermis

The dermis is about 0.1–0.5 cm thick and consists of collagenous (70 %) and elastin fibres. In the dermis, glycosaminoglycans or acid mucopolysaccharides, are covalently linked to peptide chains to form proteoglycans, the ground substance that promotes the elasticity of the skin. The main cells present are the fibroblasts, which produce the connective tissue

components of collagen, laminin, fibronectin and vitronectin; mast cells, which are involved in the immune and inflammatory responses; and melanocytes involved in the production of the pigment melanin [51]. Nerves, blood vessels and lymphatic vessels are also present in the dermis.

Contained within the dermis is an extensive vascular network providing for the skin nutrition, repair, and immune responses for the rest of the body, heat exchange, immune response, and thermal regulation. Skin blood vessels derive from those in the subcutaneous tissues (hypodermis), with an arterial network supplying the papillary layer, the hair follicles, the sweat and apocrine glands, the subcutaneous area, as well as the dermis itself. These arteries feed into arterioles, capillaries, venules, and, thence, into veins. Of particular importance in this vascular network is the presence of arteriovenous anastomoses at all levels in the skin. These arteriovenous anastomoses, which allow a direct shunting of up to 60% of the skin blood flow between the arteries and veins, thereby avoiding the fine capillary network, are critical to the skin's functions of heat regulation and blood vessel control. Blood flow changes are most evident in the skin in relation to various physiological responses and include psychological effects, such as shock (''draining of color from the skin'') and embarrassment (''blushing''), temperature effects, and physiological responses to exercise, hemorrhage, and alcohol consumption.

The lymphatic system is an important component of the skin in regulating its interstitial pressure, mobilization of defense mechanisms, and in waste removal. It exists as a dense, flat meshwork in the papillary layers of the dermis and extends into the deeper regions of the dermis. Also present in the dermis are a number of different types of nerve fibers supplying the skin, including those for pressure, pain, and temperature [52]. Epidermal appendages such as hair follicles and sweat glands are embedded in the dermis [53].

2.3. Hypodermis

The deepest layer of the skin is the subcutaneous tissue or hypodermis. The hypodermis acts as a heat insulator, a shock absorber, and an energy storage region. This layer is a network of fat cells arranged in lobules and linked to the dermis by interconnecting collagen and elastin fibers. As well as fat cells (possibly 50% of the body's fat); the other main cells in the hypodermis are fibroblasts and macrophages. One of the major roles of the hypodermis is to carry the vascular and neural systems for the skin. It also anchors the skin to underlying muscle. Fibroblasts and adipocytes can be stimulated by the accumulation of interstitial and lymphatic fluid within the skin and subcutaneous tissue [54]. The total thickness of skin is about 2–3 mm, but the thickness of the stratum corneum is only about 10–15 μm.

2.4. Skin appendages

There are four skin appendages: the hair follicles with their associated sebaceous glands, eccrine and apocrine sweat glands, and the nails, but these occupy only about 0.1 % of the total human skin surface.

The pilosebaceous follicles have about 10 to 20 % of the resident flora and cannot be decontaminated by scrubbing. The hair follicles are distributed across the entire skin surface with the exception of the soles of the feet, the palms of the hand and the lips. A smooth muscle, the erector pilorum, attaches the follicle to the dermal tissue and enables hair to stand up in response to fear. Each follicle is associated with a sebaceous gland that varies in size from 200 to 2000 μm in diameter. The sebum secreted by this gland consisting of triglycerides, free fatty acids, and waxes, protects and lubricates the skin as well as maintaining a pH of about 5. Sebaceous glands are absent on the palms, soles and nail beds. Sweat glands or eccrine glands respond to temperature via parasympathetic nerves, except on palms, soles and axillae, where they respond to emotional stimuli via sympathetic nerves [51]. The eccrine glands are epidermal structures that are simple, coiled tubes arising from a coiled ball, of approximately 100 μm in diameter, located in the lower dermis. It secretes a dilute salt solution with a pH of about 5, this secretion being stimulated by temperature-controlling determinants, such as exercise and high environmental temperature, as well as emotional stress through the autonomic (sympathetic) nervous system. These glands have a total surface area of about 1/10,000 of the total body surface. The apocrine glands are limited to specific body regions and are also coiled tubes. These glands are about ten times the size of the eccrine ducts, extend as low as the subcutaneous tissues and are paired with hair follicles.

Nail function is considered as protection. Nail plate consists of layers of flattened keratinized cells fused into a dense but elastic mass. The cells of the nail plate originate in the nail matrix and grow distally at a rate of about 0.1 mm/day. In the keratinization process the cells undergo shape and other changes, similar to those experienced by the epidermal cells forming the stratum corneum. This is not surprising because the nail matrix basement membrane shows many biochemical similarities to the epidermal basement membrane [55,56]. Thus, the major components are highly folded keratin proteins with small amounts of lipid (0.1–1.0%). The principal plasticizer of the nail plate is water, which is normally present at a concentration of 7–12 %.

3. Skin functions

Many of the functions of the skin can be classified as essential to survival of the body bulk of mammals and humans in a relatively hostile environment. In a general context, these functions can be classified as a protective, maintaining homeostasis or sensing. The importance of the protective and homeostatic role allows the survival of humans in an environment of variable temperature; water content (humidity and bathing); and the presence of environmental dangers, such as chemicals, bacteria, allergens, fungi and radiation. In a second context, the skin is a major organ for maintaining the homeostasis of the body, especially in terms of its composition, heat regulation, blood pressure control, and excretory roles. It has been argued that the basal metabolic rate of animals differing in size should be scaled to the surface area of the body to maintain a constant temperature through the skin's thermoregulatory control [57]. Third, the skin is a major sensory organ in terms of

sensing environmental influences, such as heat, pressure, pain, allergen, and microorganism entry. Finally, the skin is an organ that is in a continual state of regeneration and repair. To fulfill each of these functions, the skin must be tough, robust, and flexible, with effective communication between each of its intrinsic components mentioned above.

The stratum corneum also functions as a barrier to prevent the loss of internal body components, particularly water, to the external environment. The epidermis plays a role in temperature, pressure, and pain regulation.

Appendage functions are following: hair follicle and sebaceous gland fulfill with protect (hair) and lubricate (sebum), eccrine and apocrine glands have the functions of cooling and vestigial secondary sex gland, respectively; and nails has the function of to protect.The hypodermis acts as a heat insulator, a shock absorber and an energy storage region. One of the major roles of the hypodermis is to carry the vascular and neural systems for the skin.

4. Routes of drug penetration through the skin

The determination of penetration pathways of topically applied substances into the skin is the subject of several investigations. The permeation of drugs through the skin includes the diffusion through the intact epidermis y through the skin appendages. These skin appendages are hair follicles and sweat glands which form shunt pathways through the intact epidermis, occupying only 0.1% of the total human skin [58]. It is known drug permeation through the skin is usually limited by the stratum corneum. Two pathways through the intact barrier may be identified, the intercellular and transcellular route (**Figure 2**):

a. The intercellular lipid route is between the corneocytes.

Interlamellar regions in the stratum corneum, including linker regions, contain less ordered lipids and more flexible hydrophobic chains. This is the reason of the non-planar 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 [59,60]. The hydrophilic molecules diffuse predominantly “laterally” along surfaces of the less abundant, water filled inter-lamellar 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 [61].

b. The transcellular route contemplates the crossing through the corneocytes and the intervening lipids [24].

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 [62]. The narrow aqueous transepidermal pathways have been observed using confocal laser scanning microscopy (CLSM). 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 better sealed and more transport resistant is the intra-cluster/inter-corneocyte pathway [63]. Hydrophilic conduits have openings between ≥5 μm (skin appendages) and ≤10 nm (narrow inter-corneocyte pores). So sweat ducts (≥50 μm), pilosebaceous units (5–70 μm), and sebaceous glands (5–15 μm) represent the largest width/lowest resistance end of the range. Junctions of corneocytes-clusters and cluster boundaries fall within the range [64]. It was determined that the maximally open hydrophilic conduits across skin are approximately 20–30 nm wide, including pore penetrant/opener thickness [63]. Another studies revealed the width of the negatively charged hydrophilic transepidermal pores expanded by electroosmosis to be around of 22–48 nm [65]. Lipophilic cutaneous barrier is governed by molecular weight and distribution coefficient rather than molecular size [66]. The relative height of cutaneous lipophilic barrier consequently decreases with lipophilicity of permeant, but molecules heavier than 400–500 Da are so large permeants to find sufficiently wide defects in the intercellular lipidic matrix to start diffusing through the lipidic parts of cutaneous barrier [64, 66,67].

The contribution to transdermal drug transport can increases with the pathways widening or multiplication, for example such that is caused by exposing the stratum corneum to a strong electrical (electroporation/iontophoresis), mechanical (sonoporation/sonophoresis), thermal stimulus, or suitable skin penetrants [59].

Recently, follicular penetration has become a major focus of interest due to the drug targeting to the hair follicle is of great interest in the treatment of skin diseases. However due to follicular orifices only occupying 0.1% of the total skin surface area, it was assumed as a non important route. But a variety of studies shown the hair follicles as could be a way to trough the skin [68- 73]. Such follicular pathway also has been proposed for topical administration of nanoparticles and microparticles and it has been investigated in porcine skin, because in recent studies the results have confirmed the *in vitro* penetration into the porcine hair follicles might be considered similar to those on humans *in vivo.* After topical application of dye sodium fluorescein onto porcine skin mounted in Franz diffusion cells with the acceptor compartment beneath the dermis, the fluorescence was detected on the surface, within the horny layer, and in most of the follicles confirming the similarity in the penetration between porcine and human skin [72]. So nanoparticles have been studied in porcine skin revealing in the surface images 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 [74]. In other investigations, it has been shown by differential stripping the influence of size microparticles in the skin penetration. It can act as efficient drug carriers or can be utilized as follicle blockers to stop the penetration of topically applied substances [73].

It has already been postulated that certain molecules can hydrogen bond to groups present on the surfaces of follicular pores [75]. However, more studies have to be made in order to identify all the molecular properties that influence drug penetration into hair follicles.

Nowadays, there are currently a number of methods available for quantifying drugs localized within the skin or various layers of the skin. To date, a direct, non-invasive quantification of the amount of topically applied substance penetrated into the follicles had not been possible. Therefore, stripping techniques, tape stripping and cyanoacrylate skin surface biopsy have been used to remove the part of the stratum corneum containing dye topically applied [76]. Thus, the "differential stripping" has been shown as a new method that can be used to study the penetration of topically applied substances into the follicular infundibula non-invasively and selectively [20,26,30,76,77].

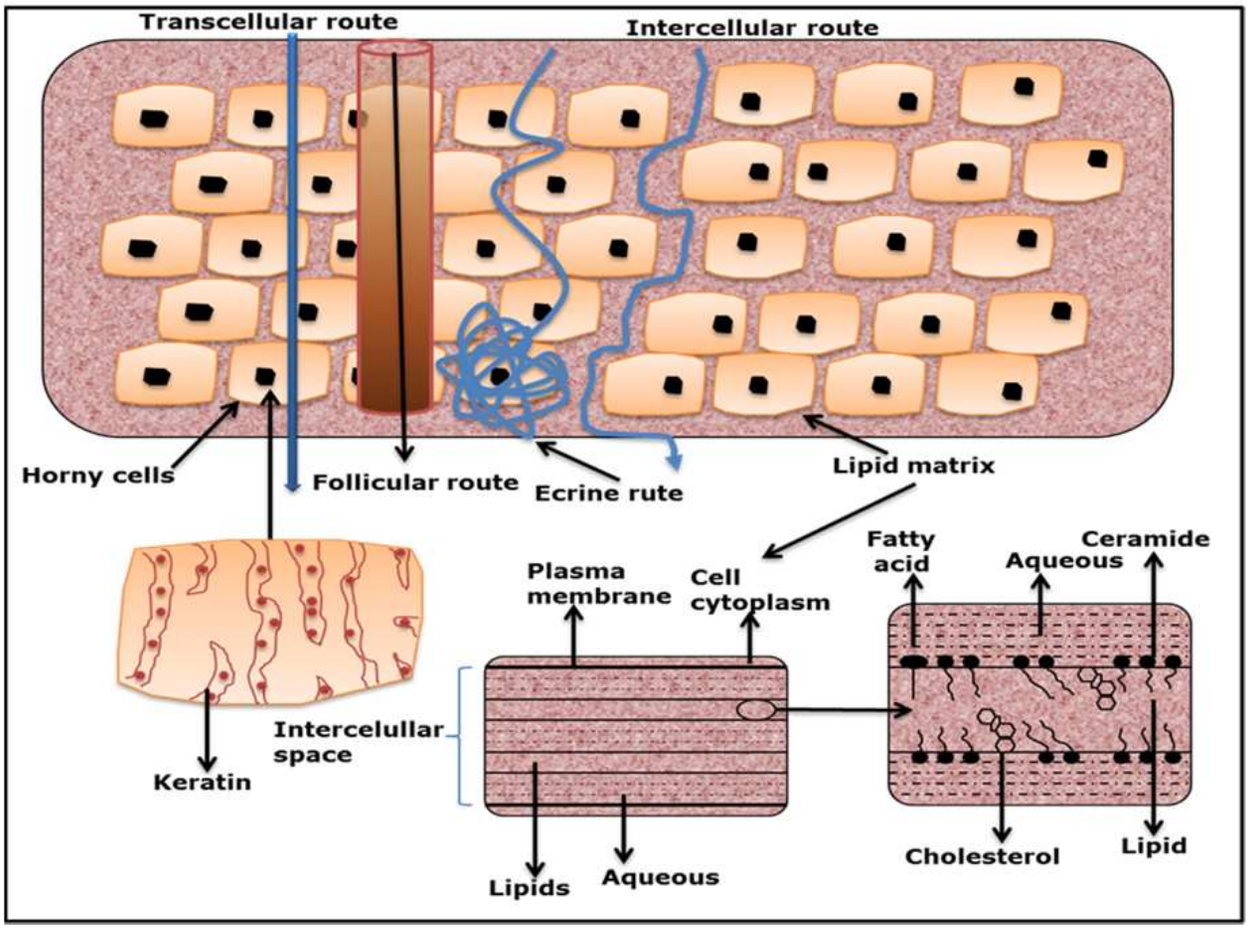

Figure 2. Schematic representation of penetration routes of drugs throughout the skin.

5. Advantages and disadvantages of transdermal drug delivery

Transdermal drug delivery systems offer several important advantages over more traditional approaches, in addition to the benefits of avoiding the hepatic first-pass effect, and higher patient compliance, the additional advantages and the disadvantages [78-80] that transdermal drug delivery offers can be summarized as follows in Table 1.

Advantages of transdermal drug delivery	Disadvantages of transdermal drug delivery
Longer duration of action	Possibility of local irritation at the site of application
Reduction in dosing frequency	Erythema, itching, and local edema can be caused by the drug, the adhesive, or other excipients in the patch formulation
More uniform plasma levels	The skin's low permeability limits the number of drugs that can be delivered in this manner
Useful for drugs that require relatively consistent plasma levels	
It is an alternative route of administration to accommodate patients who cannot tolerate oral dosage forms (specially for nauseated or unconscious patients)	
Improved bioavailability	
Reduction of side effects	
Flexibility of terminating the drug administration by simply removing the patch from the skin	

Table 1. Main advantages and disadvantages of transdermal drug delivery

6. Nanocarrier systems

Nanocarriers have demonstrated increased drug absorption, penetration, half-life, bioavailability, stability, etc. Nanocarriers are so small to be detected by immune system and they can deliver the drug in the target organ using lower drug doses in order to reduce side effects. Nanocarriers can be administrated into the organisms by all the routes; one of them is the dermal route. The nanocarriers most used and investigated for topical/transdermal drug delivery in the pharmaceutical field are shown in **Figure 3** and **Table 2**.

6.1. Nanoparticles

Nanoparticles are smaller than 1,000 nm. Nowadays, it is possible to insert many types of materials such as drugs, proteins, peptides, DNA, etc. into the nanoparticles. They are constructed from materials designed to resist pH, temperature, enzymatic attack, or other problems [81]. Nanoparticles can be classified as nanospheres or nanocapsules (See **Figure**

4). Nanospheres are solid-core structures and nanocapsules are hollow-core structures. Nanoparticles can be composed of polymers, lipids, polysaccharides and proteins [82,83]. Nanoparticles preparation techniques are based on their physicochemical properties. They are made by emulsification-diffusion by solvent displacement, emulsification-polymerization, in situ-polymerization, gelation, nanoprecipitation, solvent evaporation/extraction, inverse salting out, dispersion polymerization and other derived from these one.

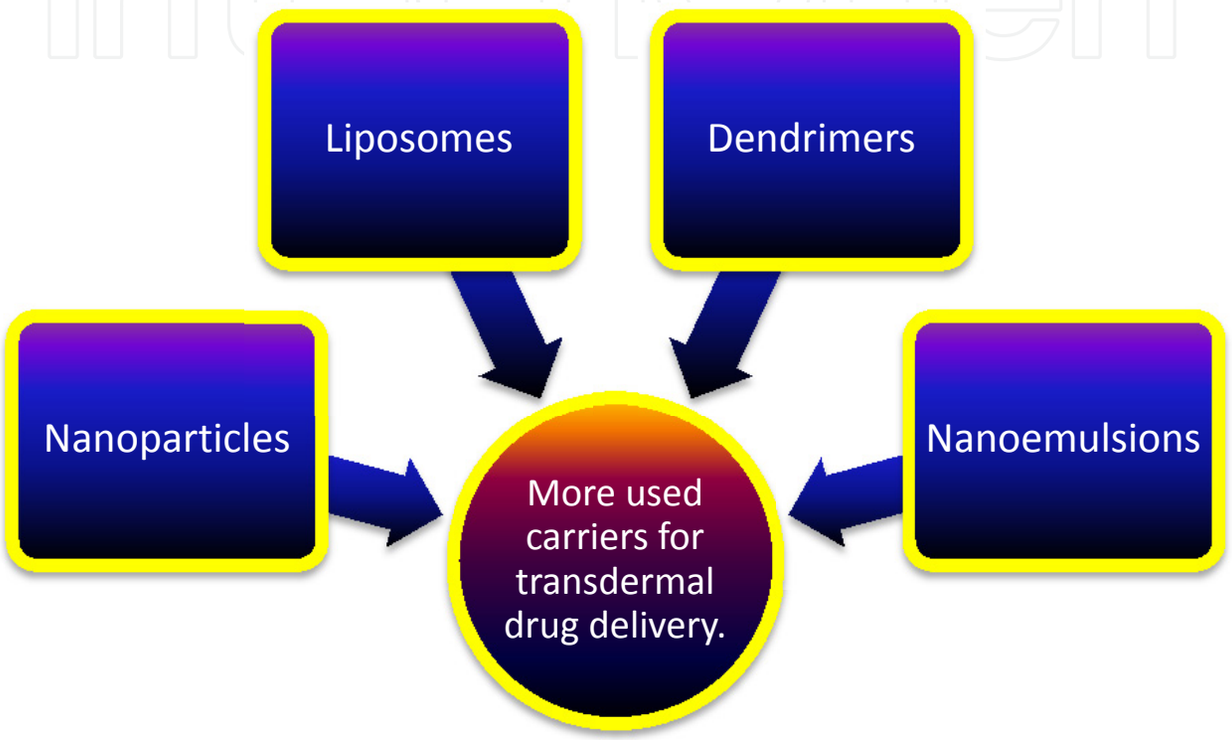

Figure 3. More used transdermal nanocarriers

6.2. Nanoemulsions

Nanoemulsions are isotropic dispersed systems of two non miscible liquids, normally consisting of an oily system dispersed in an aqueous system (o/w nanoemulsion), or an aqueous system dispersed in an oily system but forming droplets or other oily phases of nanometric sizes (100 nm). They can be stable (methastable) for long times due to the extremely small sizes and the use of adequate surfactants. Nanoemulsions can use hydrophobic and hydrophilic drugs because it is possible to make both w/o or o/w nanoemulsions [84]. They are non-toxic and non-irritant systems and they can be used for skin or mucous membranes, parenteral and non parenteral administration in general and they have been used 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 been reduced due to the stability problems inherent to this dosage form.

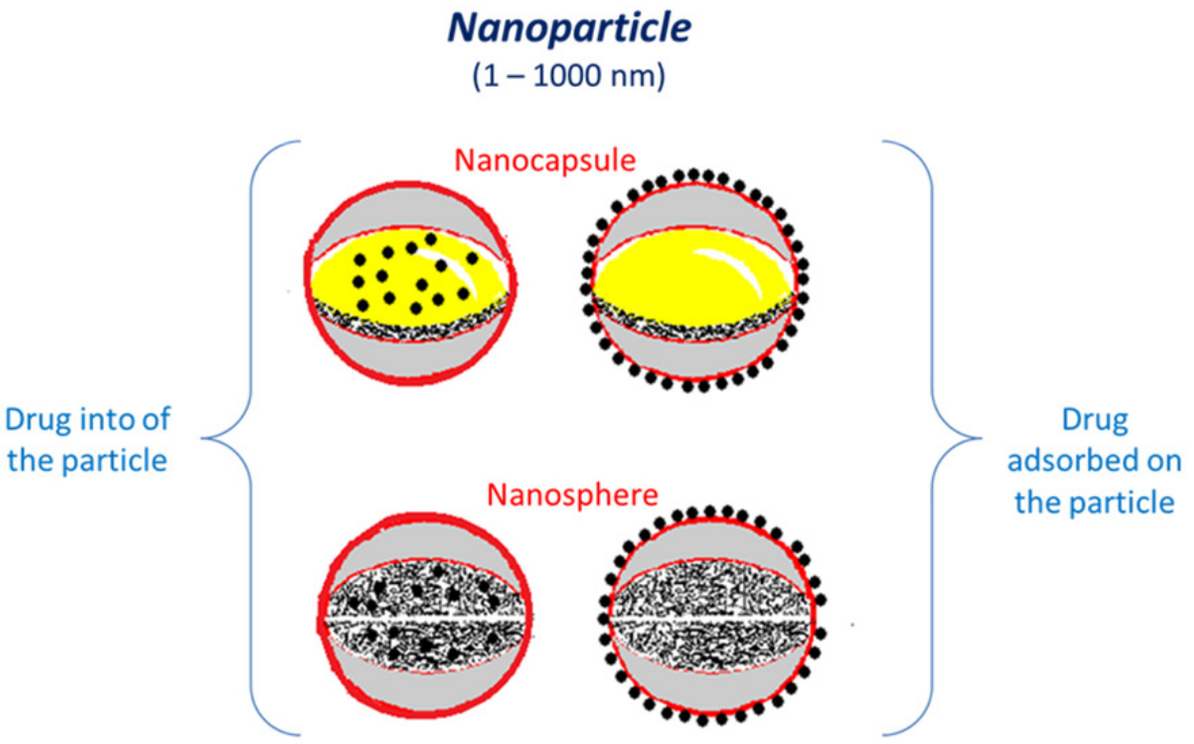

Figure 4. Nanospheres and nanocapsules are small vesicles used to transport drugs. Nanospheres are typically solid polymers with drugs embedded in the polymer matrix. Nanocapsules are a shell with an inner space loaded with the drug of interest. Both systems are useful for controlling the release of a drug and protecting it from the surrounding environment.

6.3. Liposomes

Liposomes are hollow lipid bilayer structures (**Figure 5**) that can transport hydrophilic drugs inside the core and hydrophobic drugs between the bilayer [85]. They are structures made of cholesterol and phospholipids. They can have different properties depending on the excipients included and the process of their elaboration. The nature of liposomes makes them one of the best alternatives for drug delivery because they are non-toxic and remain inside the bloodstream for a long time. Liposomes can be surface-charged as neutral, negative or positive, depending on the functional groups and pH medium. Liposomes can encapsulate both lipophilic and hydrophilic drugs in a stable manner, depending on the polymer added to the surface [86]. There are small unilamellar vesicles (25 nm to 100nm), medium-sized unilamellar vesicles (100 nm and 500nm), large unilamellar vesicles, giant unilamellar vesicles, oligolamellar vesicles, large multilamellar vesicles and multivesicular vesicles (500 nm to microns). The thickness of the membrane measures approximately 5 to 6 nm. These shapes and sizes depend of the preparation technique, the lipids used and process variables. Depending on these parameters, the behavior both in vivo and in vitro can change and opsonization processes, leakage profiles, disposition in the body and shelf life are different due to the type of liposome [86].

Liposomes preparation techniques follow three basic steps with particular features depending on safety, potential scale up and simplicity: 1) Lipid must be hydrated, 2) Liposomes have to be sized and 3) Nonencapsulated drug has to be removed. The degree of transdermal drug penetration is affected by the lamellarity, lipid composition, charge on the liposomal surface, mode of application and the total lipid concentrations [87].

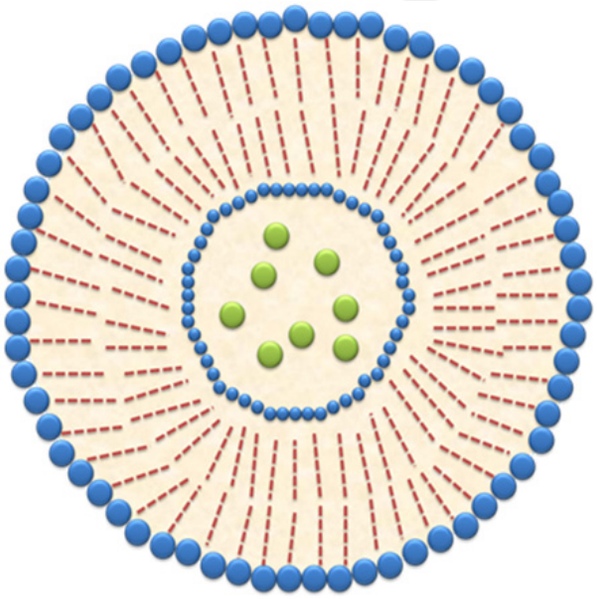

Figure 5. Liposomes are spherical vesicles that comprise one or more lipid bilayer structures enclosing an aqueous core. They protect encapsulated drugs from degradation. Liposomes can also be functionalized to improve cell targeting and solubility

6.4. Dendrimers

Dendrimers are monodisperse populations that are structurally and chemically uniform (**Figure 6**). They allow conjugation with numerous functional groups due to the nature of their branches. The amount of branches increases exponentially and dendrimers growth is typically about 1 nm per generation [88]. The dendrimers classification is based on the number of generations. After the creation of a core, the stepwise synthesis is called first generation; after that, every stepwise addition of monomers creates the next generation. This approach allows an iterative synthesis, providing the ability to control both molecular weight and architecture.

The kind of polymer chosen to construct the dendrimer by polimerization is very important with regard to the final architecture and features. In addition, the use of branched monomers has the peculiarity of providing tailored loci for site-specific molecular recognition and encapsulation. Notably, 3D and fractal architecture, as well as the peripheral functional groups, provide dendrimers with important characteristic physical and chemical properties. In comparison with linear polymers, dendritic structures have "dendritic voids" that give these molecules important and useful features. These spaces inside dendrimers can mimic the molecular recognition performed by natural proteins.

Furthermore, dendrimers have a high surface-charge density due to ionizable groups that help them to attach drugs by electrostatic forces, regardless of the stoichimetry. This dendrimer-drug association provides drugs with better solubility, increasing their transport through biological membranes and sometimes increasing drug stability. The number of molecules that can be incorporated into dendrimers is related to the number of surface functional groups; therefore, later-generation dendrimers are more easily incorporated into dendritic structure. However, not all the functional groups are available for interaction due to steric volume, molecule rotation or stereochemistry effects. Dendrimers can have positive and negative charges, which allows them to complex different types of drugs [89].

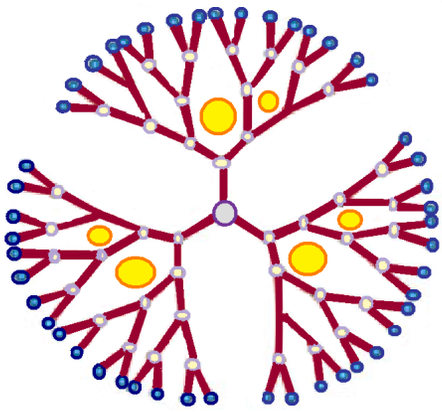

Figure 6. Dendrimers are highly branched polymers with a controlled three-dimensional structure around a central core. They can accommodate more than 100 terminal groups.

6.5. Advantages and limitations of using nanocarriers for transdermal drug delivery

As it is has been mentioned before, the search for new strategies able to enhance the topical and transdermal penetration of drugs has become essential [61]. Different carrier systems have been proposed in an attempt to favour the transport of drugs through the skin, enabling drug retention and in some cases allowing a controlled release. Skin penetration is essential to a number of current concerns, for example, contamination by microorganisms and chemicals, drug delivery to skin (dermatological treatments) and through skin (transdermal treatments), and skin care and protection (cosmetics) [103-106].

Follicular penetration has become a major focus of interest due to the drug targeting to the hair follicle is of great interest in the topical treatment of skin diseases. However due to follicular orifices only occupying ~0.1% of the total skin surface area, it was assumed as a non important route. But recently, a variety of studies have shown that the hair follicles represent a important way to trough the skin and some techniques have been used to test the penetration of drugs loaded nanoparticles/microsparticles [70,72,73,107-109]. Confocal laser scanning microscopy which permits optical sectioning of thick tissues and cells and their subsequent computerized three-dimensional reconstruction, has been used to study the entry of drugs through the skin [110]. It was visualized in the fresh human scalp skin

on-line the diffusion processes of a model fluorophore into the hair follicle at different depths [72]. Such follicular pathway also has been proposed for topical administration of nanoparticles and microparticles using porcine skin. Recent studies have confirmed that the *in vitro* penetration into the porcine hair follicles might be considered similar to those on humans *in vivo* [73]. Studies in porcine skin revealed in the surface images 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 [76]. In other investigations, it has been shown by differential stripping the influence of size microparticles in the skin penetration. It can act as efficient drug carriers or can be utilized as follicle blockers to stop the penetration of topically applied substances [75]. An alternative technique is multiphoton microscopy (MPM) especially two-photon excitation microscopy has been widely used in imaging biological specimens treated with nanoparticles [111-114]. The near-infrared light used in the two-photon microscope can penetrate deeper in highly scattering tissues such as *in vivo* human skin than confocal microscopes operated with ultraviolet excitation [115]. Futhermore, this technique provides both cellular and extracellular structural information, with subcellular resolution helpful for clinical dermatological diagnosis, both *ex vivo* and *in vivo*. In addition, it can be used to characterize stratum corneum structures, visualize and quantify transcutaneous drug delivery, detect skin cancers, explore collagen structural transitions, and watch laser–skin interactions [116,117]. A common method used for quantifying drugs localized within the skin or various layers of the skin are the tape stripping and cyanoacrylate skin surface biopsy techniques, which have been used to remove the part of the stratum corneum containing dye topically applied. Thus, the "differential stripping" has been shown as a new method that can be used to study the penetration of topically applied substances into the follicular infundibula non-invasively and selectively [118]. It has been reported in a previous *in vitro* permeation studies using tape stripping, that triclosan-loaded nanoparticles penetrated into the skin and their retention favoured a local effect. Moreover, polymeric nanoparticles are expected to be able to form a depot in the hair follicles, providing a targeted controlled drug delivery [33,119].

In general, the principal advantages of microparticles and nanoparticles over conventional formulations such as creams, solutions, ointments, lotions, gels, and foams, is that the second ones have different absorption characteristics and aesthetic properties, and they also have some major limitations, such as poor penetration and uncontrolled drug release. Furthermore, tolerability and safety end points, such as irritation, dryness, erythema, itching, stinging and burning will be key factors in determining its usefulness. It happens because using a traditional system, drug delivery is sometimes rapid and topical or plasmatic concentrations can result in toxic effects. However, for the case of nanoparticles a smaller amount of the drug is necessary and due to the targeted nature of delivery [120]. Topical or transdermal drug deliveries have many advantages over the other routes: fewer side effects, increased patient compliance, controlled release, and the lack of a hepatic first pass [121].

Nanocarrier	Size range	Preparation methods	Characteristics	References
Polymeric nanoparticles	10-1000 nm	-In situ polymerization -Emulsification-evaporation, -Emulsification-diffusion, -Emulsification-diffusion by solvent displacement.	Solid or hollow particles wich have entraped, binded or encapsulated drugs.	[33]
Solid lipid nanoparticles	50-1000 nm	-High-pressure homogenization.	Similar to polymeric nanoparticles but made of solid lipids.	[90]
Inorganic Nanoparticles	<50nm	-Sol-gel technique	Nanometric particles, made up of inorganic compounds such as silica, titania and alumina.	[91]
Liposomes	25 nm-100 μm	-Sonication -Extrusion, -Mozafari method	Vesicles composed of one or more concentric lipid bilayers, separated by water or aqueous buffer compartments.	[92]
Dendrimers	3–10 nm	-Polymerization	Macromolecular high branched structures.	[93]
Quantum dots	2-10nm	-Colloidal assembly, viral assembly, -Electrochemical assembly.	Made up of organic surfactants, precursors and solvents.	[94]
Lipid globules	1-100 nm	-Emulsification espontaneous systems.	Multicomponent fluid made of water, a hydrophobic liquid, and one or several surfactants resulting in a stable system.	[95]
Lipid microcylinders	<1 μm	-Self emulsification	Self organizing system in which surfactants crystallize into tightly packed bilayers that spontaneously form cylinders.	[96]

Ethosomes	<400 nm	-Cold method -Hot method	Non invasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation.	[97]
Aquasomes	60-300 nm	-Self-assembling of hydroxyapatite by coprecipitation method	The particle core is composed of noncrystalline calcium phosphate or ceramic diamond, and it is covered by a polyhydroxyl oligomeric film.	[98]
Pharmacosomes	<200 nm	-Hand-shaking method -Ether-injection method	Pure drug vesicles formed by amphiphilic drugs.	[99]
Colloidosomes	200nm – 1.5 μm	-Self-assembly of colloidal particles at the interface of emulsion droplets	Hollow capsules with elastic shells.	[100]
Niosomes	10-1000 nm	-Self-assembly of nonionic Surfactant	Bilayered structures made of non-ionic surfactant vesicles.	[101]
Nanoemulsions	20-200nm	-High-pressure homogenization. -Microfluidization. -Phase Inversion temperature	Submicron emulsions o/w or w/o.	[102]

Table 2. Examples of nanocarriers used for drug delivery.

It has been reported that nanoencapsulation of drugs (nano-medicines) increases their efficacy, specificity, tolerability and therapeutic index [122-124]. These nano-formulations are reported to be superior to traditional medicine with respect to controlled release, targeted delivery and therapeutic impact. The targeting capabilities of nanomedicines are influenced by particle size, surface charge, surface modification, and hydrophobicity. Of these, nanoparticle size distribution is an important factor in determining the interaction with the cell membrane and their penetration across physiological barriers, being dependent on the tissue, target site and circulation [125]. Example of this are the nanostructured lipid

carriers (NLC) by their structure (lipid nanoparticles with solid matrix) increase in loading capacity, physical and chemical long-term stability, triggered release and potentially supersaturated topical formulations with respect to solid lipid nanoparticles (SLN). Other advantages of NLC include improvement in stabilisation of incorporated compounds, controlled release, occlusivity, film formation on skin including in vivo effects on the skin. Lipid nanoparticles have been observed as a good option for transdermal delivery because they can be prepared in different sizes and it is possible to modify surface polarity in order to improve skin penetration [126,127]. From the upper skin, nanoparticles can reach deeper skin regions because they exhibit mechanical flexion [128].

Additionaly, transdermal nanocarriers are able to reach target organs because they can be attached to antibodies, antigens, vitamins and other molecules to be more specific. Nanoparticles can travel largely undetected by the immune system depending of the nanocarriers size of the antigen added as well as its composition. So, by hiding functional groups or protecting these groups with other molecules, drugs can be released specifically in the target organ. Consecuently, nanoparticles can even travel from the skin to lymph nodes, representing a promising tool for immunomodulation [129]. One of the first strategies for transdermal delivery were the liposomes. The nature of liposomes makes them one of the best alternatives for drug delivery because they are non-toxic and remain inside the bloodstream for a long time [130-133]. Nevertheless, some factors affect the degree of transdermal drug penetration such as the lamellarity, the lipid composition, the charge on the liposomal surface, the mode of application and the total lipid concentrations [89,134]. For that reason, flexible vesicles called transfersomes or transformable liposomes have been compared with those rigid vesicles to enhance penetration [135-142]. The lipids present in the liposome bilayer can interact with lipids present in the stratum corneum changing the structure of the upper skin. This change is beneficial for the penetration of lipophilic drugs into the stratum corneum [143]. Some liposomes may have a deformable structure and pass through the stratum or may accumulate in the channel-like regions in the stratum corneum, depending upon their composition [144,145]. In order to obtain transformable liposomes more flexible, they are prepared using surfactants or alcohol (ethosomes) in the lipid bilayer, to be able to deform them when a pressure is applied in the transdermal route.

Some limitations for nanocarriers are the important tests and regulations that should be carried out to ensure an adequate characterization, analytical evaluation, toxicological and pharmacological assessment, which is necessary to determine the efficacy of using these nanostructures in therapies and diagnosis because of their tiny size, their high surface energy, their composition, their architecture, their attached molecules, etc. Those things are frequently reviewed for the dendrimers. One of the main advantages is that they have multivalency and it is possible to get control of the functional groups on the surface [146,147]. Due to their form and size (1–10 nm), these molecules can carry drugs, imaging agents, and can interact with lipids present in membranes, because it was reported a better permeation in cell cultures and intestinal membranes. They also increased the permeation of lipophilic drugs instead of hydrophilic drugs. The main problems with this kind of

transdermal carrier are poor biodegradation and inherent cytotoxicity [148]. To obtain dendrimers less toxic, dendrimers have been linked to peptides. Dendrimers-peptides are formed from amino acids linked via peptide-amide bonds to the branches of dendrimers in the core or on the surface to get down the toxicity. Then, they are bio-transformed to produce amino-acid derivatives. Besides, the synthesis of these structures is less expensive and purification does not present any difficulty [149,150].

It is suggested in future research to elucidate the interactions between nanocarriers and other molecules as well as interactions between nanocarriers and biological entities. The toxicology of nanostructures is also a current concern. Materials behave very differently when they are diminished to nanosizes. Traditional laws do not work at this "meso-scale" in the same way as they function at the macro-scale. On the macro scale, bulk properties in a material predominate over surface properties. At the micro-scale, surface properties tend to dominate. At the meso-scale, both types of properties play significant roles [151,152]. Furthermore, the effects of metabolized/altered nanostructures on the biological system are difficult to predict. Regulatory agencies are taking action to assess new Nanotechnology-based products.

In addition, the fabrication of nanocarriers scaling up from the lab at the industrial production is difficult and the materials used to prepare nanocarriers are very expensive in the majority of the cases.

7. Applications of nanocarrier systems in topical/transdermal delivery

Nanocarriers as drug delivery systems were first intended for use in parenteral or oral routes of administration and as such still continue to be the focus of many studies [153]. However skin application of these nanocarriers, and especially for liposomes, polymeric and lipidic nanoparticles, also makes sense when considering surface effects (film formation and occlusive effects), local effects in the skin (drug delivery in the epidermis and dermis) and systemic effects (deeper drug permeation and transdermal delivery). In potential uses apart from those concerned with surface effects the nanocarrier has to overcome the SC barrier in order to deliver the drug more or less deeply into skin layers. Recent advances in the study of penetration mechanisms deal with the control of the intercellular penetration route by the crystalline state of lipids, and the penetration through skin appendages (the follicular pathway) that appears to contribute much more than was previously thought. Applications dependent on skin penetration that have received special attention include transdermal delivery of nano- and microparticles by hair follicles, especially for nanoparticles which penetrate hair follicles very efficiently targeting the skin immune system in order to develop new vaccination strategies, and problems relating to skin diseases [154,155].

Options for topical and transdermal delivery are the solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) [156]. Some drugs such as triptolide, triamcinolone acetonide acetate, cyclosporin A have been used to be entrapped in SLN [157-159]. SLN can be admixed to an already commercially available and established topical formulation, e.g. a

cosmetic day cream. Admixing the SLN leads to an increase in occlusivity while still maintaining the 'light character' of the day cream and avoiding the glossiness of more occlusive night creams. This phenomena is explained in **Figure 7**. However, having a highly occlusive night cream already, addition of SLN will have little or no effect [156].

Lipid nanoparticles are other options to load arthemeter and econazole nitrate [160,161]. Celecoxib [162]. It was compared the permeability of coenzima Q 10 incorporated in NLC and in an emulsion with the same lipid contain. The occlusion effect of the cream was also investigated. The result showed a higher permeability of the molecule and a higher occlusive effect for the NLC than for the emulsion as it could be observed in **Figures 8 and 9** [163].

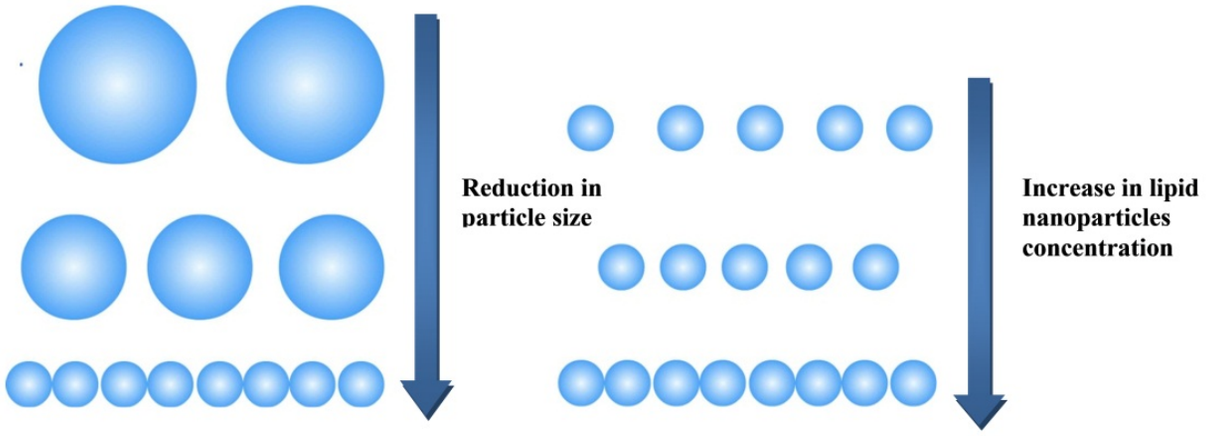

Figure 7. The occlusion factor of lipid nanoparticles depends on various factors: at identical lipid content, reducing the particle size leads to an increase in particle number, the film becomes denser (left) and therefore the occlusion factor increases. At a given particle size, increasing the lipid concentration increases particle number and density of the film (right) which also leads to a higher occlusion factor.

Different studies shown lipid nanoparticles were able to enhance the chemical stability of compounds sensitive to light, oxidation and hydrolysis. Enhancement of chemical stability after incorporation into lipid nanocarriers was proven for many cosmetic actives, e.g. coenzyme Q10 [164-166], ascorbyl palmitate [164,167], tocopherol (vitamin E) [165] and retinol (vitamin A) [168-170].

Three vitamin derivatives including vitamin C (ascorbyl tetraisoplamitate), vitamin E (tocopherol acetate) and vitamin A (retinyl palmitate) were also loaded in PLGA nanospheres, for skin whitening and anti-wrinkles/aging applications due vitamin C suppresses the blemishes because it limits the activity of tyrosinase, which promotes melanin production. Furthermore, it increases collagen formation to reduce wrinkles, and prevents cell oxidation by eliminating active oxygen. As to vitamin E and A, they also act as antioxidant and collagen promoter, respectively. They were able to reach the target areas in a stable form, sustain the pharmacological effect for a long time and be effective to reduce the wrinkles and produce a whitening effect [171]. In a sense, the idea of nanoparticle design for drug delivery systems in cosmetics applications is important.

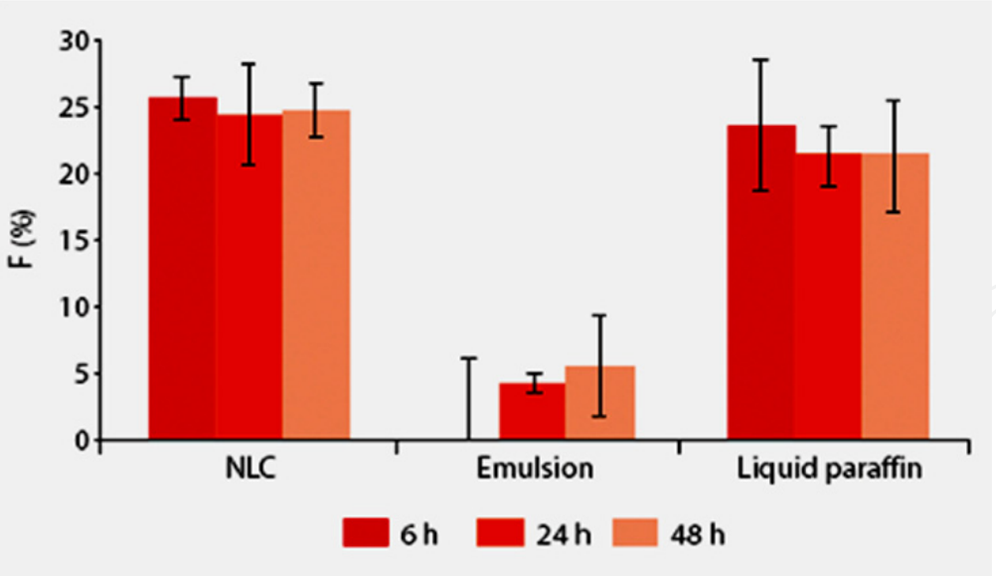

Figure 8. Occlusion factor (F) of NLC, emulsion and liquid paraffin (with permission from authors) [163].

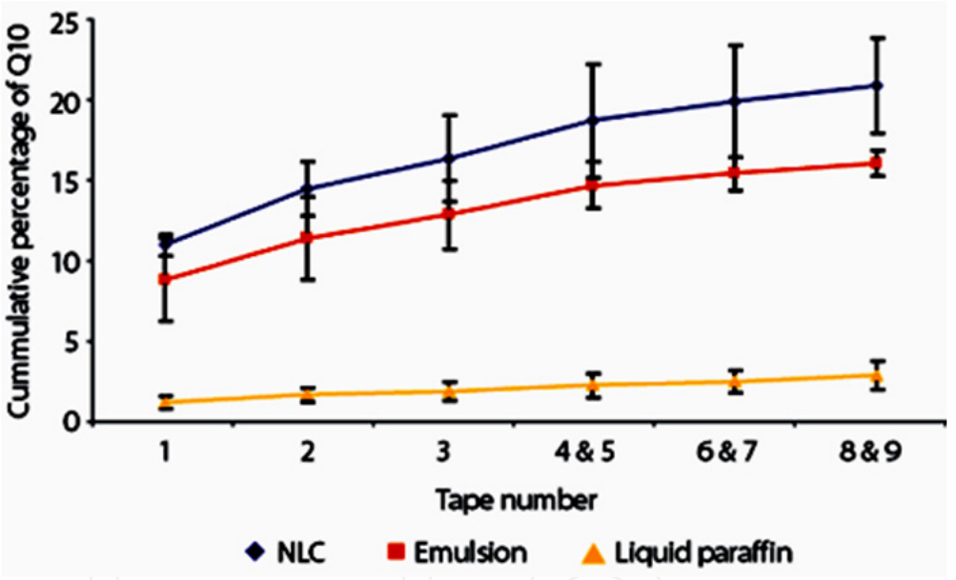

Figure 9. Tape stripping test: summation of coenzyme Q10 found in the tapes related to the applied amount (with permission from authors) [163].

As it was described before, the follicles are deep invaginations inside skin where the SC is thinner, the vascularisation is denser, and there are several targets of interest along a follicle structure from both cosmetic and pharmaceutical viewpoints [172]. Here, minoxidil which is an antihypertensive has been introduced in a block of copolymer poly (ε-caprolactone)-block-poly(ethyleneglycol) to treat the alopecia areata disorder , by widening blood vessels and opening potassium channels, it allows more oxygen, blood, and nutrients to the follicle. This disorder is an inflammatory condition, often reversible hair loss affecting mainly children and young adults. Clinically, round hairless patches appear on the scalp while hair follicles remain intact. This skin disorder is related with the distal part of the human hair follicle immune system, especially with the interacting intraepithelial T cells. The cause of

this condition is diverse and seems to involve T cell–mediated immunologic changes, neuropeptides, genetic disposition to autoimmunity, and distress [30,173,174]. As the infundibulum of the hair follicle is surrounded by an extensive capillary network and the permeability of its epithelium allows the transport of molecules or particles to the blood circulatory system. There is a high density of immune cells in and around the infundibulum epithelium which could be targets also for hair follicle immune system and topical vaccination.

The sebaceous glands associated with hair follicles provide another potential target for delivering drugs against acne, androgenetic alopecia and other sebaceous gland dysfunctions. Different nanoparticles formulations have been prepared in order to treat acne vulgaris, which is an inflammatory disease of the pilosebaceous units, most densely concentrated on the face and torso [175]. Pathogenesis is multifactorial, but *Propionibacterium acnes* a Gram-positive bacterium plays a central role in the promotion of inflammation in acne. The most commonly formulations are prepared with different topical antimicrobials, either alone or in combination with other drugs. It is expected that an agent able to inhibit *P. acnes* growth and to suppress the inflammatory response will provide significant benefits to patients with acne vulgaris [30,176,177]. For that reason triclosan has been used in several systems. It was reported the characterization of triclosan loaded polymeric nanoparticles. They showed a good encapsulation efficiency and also a good physical stability representing an alternative as a treatment of acne [33]. Triclosan loaded nanoparticles made of chitosan and cyclodextrins were prepared using a very simple ionic gelation technique. This new approach permits to enhance the entrapment of hydrophobic drugs by forming molecular inclusion complexes with cyclodextrins in aqueous media. Such a device could be of interest for conferring protection to some specific drug molecules through the complexation followed by entrapment in the polymer matrix [109]. Another drug used to treat this disorder is tretinoin (all-*trans*-retinoic acid) which is the active form of a metabolic product of Vitamin A, also called retinoic acid. Tretinoin-loaded nanocapsules improved tretinoin photostability, independently on the type of oily phase used (capric/caprylic triglycerides and sunflower seed oil) in this study, and represent a potential system to be incorporated in novel topical or systemic dosage forms containing tretinoin [178].

Formulations of nanoparticles are often used in combination with penetration chemical and physical enhancers to modify the physical state of the stratum corneum, affecting the degree of transdermal drug penetration. DNA has been entrapped in nanoparticle of polysaccharide such as chitosan/poly-γ-glutamic acid and in a multifunctional core-shell polymeric nanoparticle of PLGA core and a positively-charged glycol chitosan (GC) shell. Another drugs used in the preparation of nanoparticles made of propyl-starch derivatives are flufenamic acid, testosterone and caffeine [179,180]. Insulin is a protein which has also been introduced in chitosan nanoparticles [83]. Poly (lactide-co-glycolide) polymer has been used to prepare biodegradables nanoparticles containing dexamethasone phosphate and 5-Fluorouracil [181,182]. Chlorhexidine loaded polymeric nanoparticle are used to treat cutaneous infections [183].

Inflammatory skin diseases account for a large proportion of all skin disorders and constitute a major health problem worldwide. Psoriasis, atopic dermatitis, poison ivy, and eczema are another skin disorders. Contact dermatitis, atopic dermatitis, and psoriasis represent the most prevalent inflammatory skin disorders and share a common efferent T-lymphocyte mediated response. Oxidative stress and inflammation have recently been linked to cutaneous damage in T-lymphocyte mediated skin diseases, particularly in contact dermatitis [184]. Poison ivy and atopic dermatitis may also present with bullous and vesicular changes [185]. Lipid nanoparticles have been investigated to improve the treatments of skin diseases such as atopic eczema, psoriasis, skin mycosis and inflammations. Apart from the treatment of skin diseases by topical application, e.g. gastrointestinal side effects of non-steroidal anti-inflammatory drugs can be decreased by topical antirheumatic therapy. Drugs under investigations for dermal application using lipid nanoparticles at the present are for instance glucocorticoids, retinoids, non-steroidal anti-inflammatory drugs, COX-2 inhibitors and antimycotics. It was showed that it is possible to enhance the percutaneous absorption with lipid nanoparticles. These carriers may even allow drug targeting to the skin or even to its substructures. Thus they might have the potential to improve the benefit/risk ratio of topical drug therapy [186].

Perioral dermatitis is commonly seen in women aged 20–35 years. It presents as red papules that form superficial plaques around the perioral area, nasolabial folds and/or lower eyelids. It is minimally itchy [187,188]. Topical corticosteroids are the first-line therapy of acute exacerbations of atopic dermatitis and contact dermatitis. Prednicarbate is superior to the halogenated glucocorticoids because of an improved benefit/risk ratio. However, at the present the separation of desired anti-inflammatory effects and undesired antiproliferative effects is still not satisfying. Therefore, lipid nanoparticles were investigated as a delivery system for prednicarbate. The report show an improved extent of prednicarbate uptake by human skin *in vitro*, if applied as SLN dispersion or cream containing prednicarbate-loaded SLN. The authors found that a prednicarbate targeting to the epidermis [189]. This is particular relevant because prednicarbate in the dermis is responsible for the induction of irreversible skin atrophy while the inflammatory process is most pronounced within the epidermis [186]. Therefore, a better benefit/risk ratio is expected for the application of pretnicarbate in SLN containing topical formulations.

Tretinoin, a metabolite of vitamin A is used for topical treatment of various proliferateive and inflammatory skin diseases such as psoriasis, acne (as mentioned before), photo aging, epidermotropic T-cell lymphomas and epithelial skin cancer. One of the major disadvantages associated with the topical application of tretinoin is local skin irritation such as erythrema, peeling and burning as well as increased sensitivity to sunlight. To overcome these problems tretinoin was incorporated into SLN [190]. *In vitro* permeation studies through rat skin indicated that SLN-based tretinoin gel has a permeation profile comparable to that of the market tretinoin cream. Furthermore, Draize patch test showed that SLN-based tretinoin gel resulted in remarkably less erythremic episodes compared to the currently marketed tretinoin cream (**Figure 10**). Therefore, also for formulations containing tretinoin-loaded SLN a better benefit/risk ratio is expected.

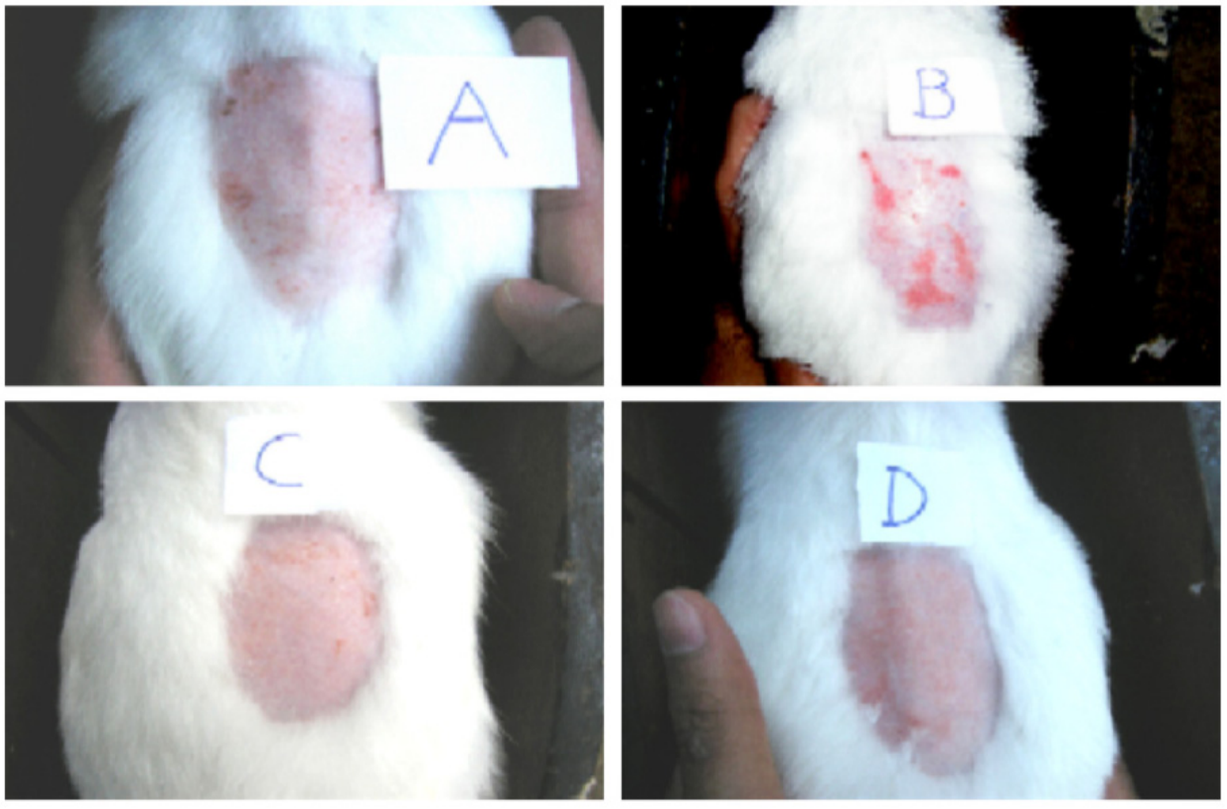

Figure 10. Pictures of Draize skin irritation studies carried out on New Zealand rabbits 24 h after application of (A) control (no application); (B) marketed formulation (Retino-A® cream); (C) SLN-based gel without tretinoin; (D) SLN-based gel containing tretinoin (0.05%, w/w). The Marketed tretinoin cream clearly shows erythemal lesions, which are not visible in SLN based tretinoin gel [190]. "Reprinted from International Journal of Pharmaceutics, 345/1-2, Kumar A. Shah, Abhijit A. Date,Medha D. Joshi,Vandana B. Patravale, Solid lipid nanoparticles (SLN) of tretinoin: Potential in topical delivery, 163-171., Copyright (2007), with permission from Elsevier"

Liposomes which were one of the first strategies for transdermal delivery are being successfully used in cancer therapy [139,141]. However to date, many liquid-type nanocosmetics 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 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. Some authors report the use of flexible vesicles called transfersomes or transformable liposomes in comparison with rigid vesicles to enhance penetration [148,191]. The application of transformable liposomes more flexible, which are prepared using surfactants or alcohol (ethosomes) in the lipid bilayer, to be able to deform them when a pressure is applied in the transdermal route has been increased.

In some researches, dendrimers are used for transdermal drug delivery. They show promising results in the delivery of drugs such as tamsulosin [192], indomethacin [193], ketoprofen and diflunisal [194] and 5-fluorouracil [195]. The main problems with this kind of transdermal carrier are poor biodegradation and inherent cytotoxicity [158]. In order to

get down the toxicity dendrimers have been linked to peptides (dendrimers-peptides) from amino acids linked via peptide-amide bonds to the branches of dendrimers in the core or on the surface [159,160].

For 5-fluorouracil (5FU) (log $P = -0.89$) which is one hydrophilic model drug used to treat skin diseases, has been reporte to have very poor penetration in skin [196-198]. Many strategies to increase skin permeation of this drug have been tested: prodrugs, terpenes, fatty acids, iontophoresis, sonophoresis, laser ablation and dendrimers which increased 5FU permeation across the skin by altering the skin structure [198-203].

Nowadays transdermal delivery using nanoemulsions it is not so used as nanoparticles or liposomes delivery due to the stability problems inherent to this dosage form. Nevertheless, Gamma Tocopherol, Caffeine, Plasmid DNA, Aspirin, Methyl Salicylate, Insulin, Nimesulide have been included in nanoemulsion. The use of these nanocarriers to deliver analgesics, corticosteroids, anti cancer agents, etc. is very important since these drugs are able to act immediately because they do not need to cross extra barriers. The drug is bioavailable easily and faster [204-210].

8. Conclusions

Nanocarriers have shown many advantages for topical and trasdermal delivery of drugs. It could be shown already for various drugs that topical/trandermal formulations containing nanoparticles can enhance the penetration into the skin increasing treatment efficiency, target the epidermis or follicles, reducing side effects. Furthermore, an increased activities as well as prolonged activities have been reported. These delivery systems can deliver both hydrophilic and lipophilic molecules. Advances with regard to materials, fabrication methods and techniques facilitate the development of new and better nanocarriers. Nonetheless, future researches must ensure the benefit and evaluate the risk ratio for many drugs included in nanocarriers.

Author details

José Juan Escobar-Chávez
Unidad de Investigación Multidisciplinaria, Laboratorio 12: Sistemas Transdérmicos y Materiales Nanoestructurados, Facultad de Estudios Superiores Cuautitlán-Universidad Nacional Autónoma de México, Estado de México

Isabel Marlen Rodríguez-Cruz
Departamento de Enseñanza e Investigación, Hospital Regional de Alta Especialidad de Zumpango. Zumpango, Estado de México

Clara Luisa Domínguez-Delgado
Departamento de Ingeniería y Tecnología. Sección de Tecnología Farmacéutica, Facultad de Estudios Superiores Cuautitlán-Universidad Nacional Autónoma de México, Estado de México

Roberto Díaz- Torres
Unidad de Investigación Multidisciplinari,. Laboratorio 9: Toxicología y genética, Facultad de Estudios Superiores Cuautitlán-Universidad Nacional Autónoma de México, Estado de México

Alma Luisa Revilla-Vázquez
Laboratorio de Desarrollo de Métodos Analíticos, Facultad de Estudios Superiores Cuautitlán-Universidad Nacional Autónoma de México, Cuautitlán Izcalli, Estado de México

Norma Casas Aléncaster
Laboratorio de Propiedades Reológicas de Alimentos, Facultad de Estudios Superiores Cuautitlán-Universidad Nacional Autónoma de México, Cuautitlán Izcalli, Estado de México

Acknowledgement

Dr. José Juan Escobar-Chávez wishes to acknowledge PAPIIT TA200-312, Cátedra PACIVE NCONS-17 and PAPIME 203612. We are also grateful with Dr. Müller and Dr. Pardeike for permission of their figures. The authors report no conflict of interests.

9. References

[1] Brower V. Is nanotechnology ready for primetime? Journal of National Cancer Institute 2006; 98:9-11.

[2] Escobar-Chávez JJ, Rodriguez Cruz IM, Dominguez-Delgado CL. Chemical and Physical enhancers for transdermal drug delivery. In: Gallelli L (ed.) Pharmacology. Rijeka: InTech; 2012. p397-433.

[3] Díaz-Torres R. Transdermal nanocarriers. In: José Juan Escobar-Chávez/Virginia Merino (Eds.) Current Technologies to Increase the Transdermal Delivery of Drugs. The Netherlands: Bentham Science Publishers; 2010. p120-40.

[4] Hadgraft J, Lane ME. Skin permeation: The years of enlightenment. International Journal of Pharmaceutics 2005, 305 (1-2):2-12.

[5] Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. European Journal of Pharmaceutical Sciences 2001; 14(2):101–4.

[6] Rizwan M, Aqil M, Talegaonkar S, Azeem A, Sultana Y, Ali A. Enhanced transdermal drug delivery techniques: an extensive review of patents. Recent Patents on Drug Delivery & Formulations 2009; 3:(2), pp. 105-24,

[7] Escobar-Chávez JJ & Merino V eds. Current Technologies to increase the Transdermal Delivery of Drugs. The Netherlands: Bentham Science Publishers; 2010.

[8] Escobar-Chávez JJ, Bonilla-Martínez D, Villegas-González A, Rodríguez-Cruz IM, Domínguez-Delgado CL. The use of sonophoresis in the administration of drugs through the skin. Journal of Pharmacy and Pharmaceutical Sciences 2009; 12(1): 88-115.

[9] Escobar-Chávez JJ, Merino V, López-Cervantes M, Rodríguez-Cruz IM, *et al*. The use of iontophoresis in the administration of nicotine and new non-nicotine drugs through the skin for smoking cessation. Current Drug Discovery Technologies 2009; 6(3):171-185.

[10] Escobar-Chávez JJ, Bonilla-Martínez D, Villegas-González A, Revilla-Vazquez AL. The electroporation as an efficient physical enhancer for transdermal drug delivery. Journal of Clinical Pharmacology 2009; 49(11):1262-83.

[11] Papakostas D, Rancan F, Sterry W, Blume-Peytavi U, Vogt A. Nanoparticles in dermatology. Archives of Dermatology Research 2011; 303(8):533-50.

[12] Kristl J, Teskac K, Grabnar PA. Current view on nanosized solid lipid carriers for drug delivery to the skin. Journal of Biomedical Nanotechnology 2010; 6(5):529-42.

[13] Schroeter A, Engelbrecht T, Neubert RH, Goebel AS. New nanosized technologies for dermal and transdermal drug delivery: A review. Journal of Biomedical Nanotechnology 2010; 6(5):511-28.

[14] Neubert RH. Potentials of new nanocarriers for dermal and transdermal drug delivery. European Journal of Pharmaceutics and Biopharmaceutics 2011; 77(1):1-2.

[15] Desai P, Patlolla RR, Singh M. Interaction of nanoparticles and cell-penetrating peptides with skin for transdermal drug delivery. Molecular Membrane Biology 2010; 27(7):247-59.

[16] Chourasia R Jain SK. Drug targeting through pilosebaceous route. Current Drug Targets 2009; 10(10):950-67.

[17] Gasco MR, Gasco P. Nanovector. Nanomedicine (London) 2007; 2(6):955-60.

[18] Magdassi S. Delivery systems in cosmetics. Colloids and Surfaces A 1997; 123-124:671-679.

[19] Luppi B, Cerchiara T, Bigucci F, Basile R, Zecchi V. Polymeric nanoparticles composed of fatty acids and polyvinylalcohol for topical application of sunscreens. Journal of Pharmacy and Pharmacology 2004;56:407-411.

[20] Kaur IP, Agrawal R. Nanotechnology: a new paradigm in cosmeceuticals. Recent Patents in Drug Delivery Formulations 2007;1:171-182.

[21] Alvarez-Roman R, Barre G, Guy RH, Fessi H. Biodegradable polymer nanocapsules containing a sunscreen agent: preparation and photoprotection. European Journal of Pharmaceutics and Biopharmaceutics 2001;52:191-195.

[22] Escobar Chávez JJ, Merino-Sanjuan V, López-Cervantes M, et al. The tape stripping technique as a method for drug quantification in skin. Journal of Pharmacy and Pharmaceutical Sciences 2008; 11(1):104-130.

[23] Potts RO, Francoeur ML. The influence of stratum corneum morphology on water permeability. Journal of Investigative Dermatology 1991; 96, 495-499.

[24] Potts RO, Guy RH. Predicting skin permeability. Pharmaceutical Research 1992; 9(5):663-669.

[25] Ellias PM. Epidermal barrier function: intercellular lamellar lipid structures, origin, composition and metabolism. Journal of Controlled Release 1991;15,199-208.

[26] Escobar-Chávez JJ, Melgoza-Contreras LM, López-Cervantes M, et al. The tape stripping technique as a valuable tool for evaluating topical applied compounds. In: Gary W. Caldwell /Atta-ur-Rahman / Z.Yan / M. Iqbal Choudhary (Eds.) Frontiers in

Drug Design & Discovery, The Netherlands: Bentham Science Publishers; 2009. Vol. 4, p189-227.

[27] Hadgraft J. Skin, the final frontier. International Journal of Pharmaceutics 2001; 224 (1-2):1-18.

[28] Guy RH and Hadgraft J. Transdermal drug delivery. New York: Marcel Dekker, Inc., p. 1-23, (2003).

[29] Nevill AM. The need to scale for differences in body size and mass: and explanation of Klieber´s 0.75 mass exponent. Journal of Applied Physiology 1994; 2870-2873.

[30] Domínguez-Delgado CL, Rodríguez-Cruz IM. & López-Cervantes M. The skin a valuable route for administration of drugs. In: José Juan Escobar-Chávez/Virginia Merino (Eds). Current Technologies To Increase The Transdermal Delivery Of Drugs. The Netherlands: Bentham Science Publishers Ltd; 2010. p1-22.

[31] Escobar-Chávez JJ, Merino V, Díez-Sales O, Nácher-Alonso A, Ganem-Quintanar A, Herráez M, Merino-Sanjuán M. Transdermal nortriptyline hydrochloride patch formulated within a chitosan matrix intended to be used for smoking cessation. Pharmaceutical Development Technology 2011, 16 (2):162-9.

[32] Escobar-Chávez JJ, López-Cervantes M, Naïk A, Kalia YN, Quintanar-Guerrero D, Ganem Quintanar A. Applications of the thermoreversible Pluronic F-127 gels in pharmaceutical formulations, Journal of Pharmacy and Pharmaceutical Sciences 2006; 9(3):339-358.

[33] Domínguez-Delgado CL, Rodríguez-Cruz IM, Escobar-Chávez JJ, Calderón-Lojero IO, Quintanar-Guerrero D, Ganem A. Preparation and characterization of triclosan nanoparticles intended to be used for the treatment of acne. European Journal of Pharmaceutics and Biopharmaceutics 2011,79(1):102-7.

[34] Fang JY, Leu YL, Wang YY, and Tsai YH. *In vitro* topical application and *in vivo* phramacodynamic evaluation of nonivamide hydrogels using Wistar rat as an animal model, European Journal of Pharmaceutical Sciences 2002;15(5):417-423.

[35] Shin SC, Cho CW,Oh IJ. Effects of non ionic surfactants as permeation enhancers towards piroxicam from the poloxamer gel through rat skins, International Journal of Pharmaceutics 2001; 222(2), 199-203.

[36] Liaw J, Lin Y-Ch. Evaluation of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) gels as a release vehicle for percutaneous fentanyl. Journal of Controlled Release 2000; 68:273-282.

[37] Wang YY, Hong CT, Chiu WT, Fang JY. *In vitro* and *in vivo* evaluations of topically applied capsaicin and nonivamide from hydrogels, International Journal of Pharmaceutics 2000; 224,1-2.

[38] Mattorano DA, Kupper LL, Nylander-French LA. Estimating dermal exposure to jet fuel (naphthalene) using adhesive tape strip samples. The Annals of Occupational Hygiene 2004; 48(2): 139–146.

[39] Chao Y-Ch, Nylander-French LA. Determination of Keratin Protein in a Tape stripped Skin Sample from Jet Fuel Exposed Skin. The Annals of Occupational Hygiene 2004; 48(1):65–73.
[40] Kenneth AW. Dermatological and transdermal formulations, New York, USA: Marcel Dekker Inc; 2002.
[41] Gray GM, White RJ, Williams RH, Yardley HJ. Lipid composition of the superficial stratum corneum cells of the epidermis. British of Journal Dermatology 1982; 106:59–63.
[42] Wertz PW, Downing DT. Ceramides of pig epidermis: structure determination. Journal of Lipid Research 1983; 24: 759-65.
[43] Long SA, Wertz PW, Strauss SJ, Downing DT. Human stratum corneum polar lipids and desquamation. Archives in Dermatology Research 1985; 277: 284-87.
[44] Wertz PW, Miethke MC, Long SA, Strauss JS, Downing DT. The composition of ceramides from human stratum corneum and from comedones. Journal of Investigative Dermatology 1985; 84: 410–12.
[45] Melnik BC, Hollmann J, Erler E, Verhoeven B, Plewig G. Microanalytical screening of all major stratum corneum lipids by sequential high-performance thin-layer chromatography. Journal of Investigative Dermatology 1989; 92: 231-34.
[46] Lavker RM, Sun T. Heterogeneity in epidermal basal keratinocytes: morphological and functional correlations. Science 1982; 215: 1239-41.
[47] Borradori L, Sonnenberg A. Structure and function of hemidesmosomes: more than simple adhesion complexes. Journal of Investigative Dermatology 1999; 112:411-18.
[48] Sawamura D, Li K, Chu M–L, Uitto J. Human bullous pemphigoid antigen (BPAG1): amino acid sequences deduced from cloned cDNAs predict biologically important peptide segments and protein domains. Journal of Biological Chemistry 1991; 266: 17784-90.
[49] Li K, Tamai K, Tan EML, Uitto J. Cloning of type XVII collagen. Complementary and genomic DNA sequences of mouse 180-kDa bullous pemphigoid antigen (BPAG2) predict an interrupted collagenous domain, a transmembrane segment, and unusual features in the 5′-end of the gene and the 3′-untranslated region of mRNA. Journal of Biological Chemistry 1993; 268: 8825-34.
[50] Stepp MA, Spurr–Michaud S, Tisdale A, Elwell J, Gipson IK. α6β4 Integrin heterodimer is a component of hemidesmosomes. Proceedings of the National Academy of Sciences 1990; 87: 8970-74.
[51] Burgeson RE, Christiano AM. The dermal–epidermal junction. Current Opinion in Cell Biology 1997; 9:651–58.
[52] Cross SE, Roberts MS. Subcutaneous absorption kinetics of interferon and other solutes. Journal of Pharmacy and Pharmacology 1993; 45: 606-09.
[53] Melski JW. The anatomy and physiology of the skin. In:Principles and Practice of Skin Excisions. Amsterdam: IEJEMEC & GBEJEMEC (eds), 1996; pp. 1–14.
[54] Steinstrasser I, Merkle HP. Dermal metabolism of topically applied drugs: Pathways and models reconsidered. Pharmaceutica Acta Helvetiae 1995; 70:3–24.

[55] Szuba A, Rockson SG. Lymphedema: anatomy, physiology and pathogenesis. Vascular Medicine 1997; 2: 321-26.
[56] Cameli N, Picardo M, Perrin C. Expression of integrins in human nail matrix. British Journal of Dermatology 1994; 130: 583–88.
[57] Philpott MP. Defensins and acne. Molecular Immunology 2003; 40: 457-462.
[58] Illel B. Formulation for transfollicular drug administration: some recent advances. Critical Reviews in Therapeutic Drug Carrier Systems 1997;14: 207-19.
[59] Xiang TX, Anderson BD. Influence of chain ordering on the selectivity of dipalmitoylphosphatidylcholine bilayer membranes for permeant size and shape. Biophysical Journal 1998; 75: 2658–71.
[60] Geinoz S, Guy RH, Testa B, Carrupt PA. Quantitative structure–permeation relationships (QSPeRs) to predict skin permeation: a critical evaluation. Pharmaceutical Research 2004; 21: 83–92.
[61] Cevc G. Drug delivery across the skin. Expert Opinion on Investigational Drugs 1997; 6: 1887–37.
[62] Cevc G, Vierl U. Nanotechnology and the transdermal route. A state of the art review and critical appraisal. Journal of Controlled Release 2010; 141(3):277-99.
[63] Schätzlein A, Cevc G. Non-uniform cellular packing of the stratum corneum and permeability barrier function of intact skin: a high-resolution confocal laser scanning microscopy study using highly deformable vesicles (Transfersomes). British Journal of Dermatology 1998; 138: 583–92.
[64] Mitragotri S. Modeling skin permeability to hydrophilic and hydrophobic solutes based on four permeation pathways. Journal of Controlled Release 2003; 86: 69–92.
[65] Aguilella V, Kontturi K, Murtomiiki L, Ramírez P. Estimation of the pore size and charge density in human cadaver skin. Journal of Controlled Release 1994; 32: 249–57.
[66] Johnson ME, Blankschtein D, Langer R. Evaluation of solute permeation through the stratumcorneum: lateral bilayer diffusion as the primary transport mechanism. Journal of Pharmaceutical Science 1997; 86:1162–72.
[67] Guy R. Transdermal drug delivery. 2a ed; Marcel Dekker. New York, USA, 2003.
[68] Meidan VM, Docker M, Walmsley AD, Irwin WJ. Low intensity ultrasound as a probe to elucidate the relative follicular contribution to total transdermal absorption. Pharmeceutical Research 1998; 15: 85–92.
[69] Ogiso T, Shiraki T, Okajima K, *et al.* Transfollicular drug delivery: penetration of drugs through human scalp skin and comparison of penetration between scalp and abdominal skins *in vitro*. Journal of Drug Targeting 2002; 10:369–78.
[70] Dokka S, Cooper SR, Kelly S, Hardee GE, Karras JG. Dermal delivery of topically applied oligonucleotides via follicular transport in mouse skin. Journal of Investigative Dermatology 2005; 124: 971–75.
[71] Grams YY, Whitehead L, Lamers G, Sturman N, Bouwstra JA. Online diffusion profile of a lipophilic dye in different depths of a hair follicle in human scalp skin. Journal of Investigative Dermatology 2005; 125:775–82.

[72] Jacobi U, Toll R, Sterry W, Lademann J. Follicles play a role as penetration pathways in *in vitro* studies on porcine skin? An optical study. Laser Physics 2005; 15: 1594–98.

[73] Teichmann A, Ossadnik M, Richter H, Sterry W, Lademann J. Semiquantitative determination of the penetration of a fluorescent hydrogel formulation into the hair follicle with and without follicular closure by microparticles by means of differential stripping. Skin Pharmacology and Physiology 2006; 19: 101–5.

[74] Alvarez-Román R, Naik A, Kalia YN, Guy RH, Fessi H. Skin penetration and distribution of polymeric nanoparticles. Journal of Controlled Release 2004; 99:53–62.

[75] Essa EA, Bonner MC, Barry BW. Human skin sandwich for assessing shunt route penetration during passive and iontophoretic drug and liposome delivery. Journal of Pharmacy and Pharmacology 2002; 54: 1481–90.

[76] Escobar-Chávez JJ, López-Cervantes M, Ganem Rondero A. Conventional Methods for Cutaneous Drug Sampling. In: Narasimha Murthy (Ed.). Dermatokinetics of therapeutic agents. Boca Raton: CRC Press Taylor and Francis Group; 2011, p81-130.

[77] Teichmann A, Jacobi U, Ossadnik M, *et al.* Differential stripping: determination of the amount of topically applied substances penetrated into the hair follicles. Journal of Investigative Dermatology 2005; 125: 264–69.

[78] Prochazka AV. New developments in smoking cessation. Chest 2000;117(4) Suppl 1:169S-175S.

[79] Wilkosz MF, Bogner RH. Trandermal drug delivery. Part 1: Current status. US pharmacist 2003; 28(4).

[80] http://www.ezinearticles.com/?Transdermal-Drug-Delivery,-Transdermal-Patches&id=155961 (accessed: 1May 2012).

[81] Huang X, Du Y, Yuan H & Hu F. Preparation and pharmacodynamics of low-molecular-weight chitosan nanoparticles containing insulin. Carbohydrate Polymers 2009; 76:368-373.

[82] Goswami S, Bajpai J & Bajpai AK. Designing Gelatin Nanocarriers as a Swellable System for Controlled Release of Insulin: An In-Vitro Kinetic Study. Journal of Macromolecular Science 2010; 47:119-130.

[83] Rodríguez-Cruz IM, Domínguez-Delgado CL, Escobar-Chávez JJ, Leyva-Gómez G, Ganem-Quintanar A, Quintanar-Guerrero D. Nanoparticle infiltration to prepare solvent-free controlled drug delivery system. International Journal of Pharmaceutics 2009; 371:177-181.

[84] Sonneville-Aubrun O, Simonnet JT & Alloret FL**.** Nanoemulsions: a new vehicle for skincare products. Advances in Colloid and Interface Science 2004; 108-109:145-149.

[85] Bangham AD. Liposomes: the Babraham connection. Chemistry and Physics of Lipids 1993; 64:275-285.

[86] Rodriguez-Justo O & Moraes ÂM. Analysis of process parameters on the characteristics of liposomes prepared by ethanol injection with a view to process scale-up: Effect of temperature and batch volume. Chemical Engineering Research and Design 2011;89(6):785-792.

[87] Cevc G, Blume G. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. Biochimica et Biophysica Acta 1992; 1104:226-232.

[88] Svenson S, Tomalia DA. Dendrimers in biomedical applications – reflections on the field. Advanced Drug Delivery Reviews 2005; 57:2106-2129.

[89] Kabanov VA, Zezin AB, Rogacheva VB, Gulyaeva ZG, Zansochova MF, Joosten JGH & Brackman J. Polyelectrolyte behavior of astramol poly(propyleneimine) dendrimers. Macromolecules 1998; 31:142-5144.

[90] Almeida AJ, Souto E. Solid lipid nanoparticles as a drug delivery system for peptides and proteins. Advanced Drug Delivery Reviews 2007; 59:478-490.

[91] García-González CA, Sampaio da Sousa AR, Argemí A, López Periago A, Saurina J, Duarte CM & Domingo C. Production of hybrid lipid-based particles loaded with inorganic nanoparticles and active compounds for prolonged topical release. International Journal of Pharmaceutics 2009; 382(1-2): pp.296-304.

[92] El Maghraby GM, Barry BW & Williams AC. Liposomes and skin: From drug delivery to model membranes. European Journal of Pharmaceutical Sciences 2008; 34:203-222.

[93] Menjoge AR, Kannan RM & Tomalia DA. Dendrimer-based drug and imaging conjugates: design considerations for nanomedical applications. Drug Discovery Today 2010;15:171-185.

[94] Rzigalinski BA, Strobl JS. Cadmium-containing nanoparticles: Perspectives on pharmacology and toxicology of quantum dots. Toxicology and Applied Pharmacology 2009; 238:280-288.

[95] Dan Y, Liu H, Gao W & Chen S. Activities of essential oils from Asarum heterotropoides var. mandshuricum against five phytopathogens. Crop Protection 2010; Vol. 29, No. 295-299.

[96] Dodla MC, Bellamkonda RV. Differences between the effect of anisotropic and isotropic laminin and nerve growth factor presenting scaffolds on nerve regeneration across long peripheral nerve gaps. Biomaterials 2008; 29:33-46.

[97] Elsayed MMA, Abdallah OY, Naggar VF & Khalafallah NM. Deformable liposomes and ethosomes: Mechanism of enhanced skin delivery. International Journal of Pharmaceutics 2006; 322:60-66.

[98] Rojas-Oviedo I, Salazar-López RA, Reyes-Gasga J & Quirino-Barreda CT. Elaboration and structural analysis of aquasomes loaded with Indomethacin. European Journal of Pharmaceutical Sciences 2007; 32:223-230.

[99] Jin Y, Tong L, Ai P, Li M & Hou X. Self-assembled drug delivery systems: 1. Properties and *in vitro/in vivo* behavior of acyclovir self-assembled nanoparticles (SAN). International Journal of Pharmaceutics 2006; 309:199-207.

[100] Rossier-Miranda FJ, Schroën CGPH & Boom RM. Colloidosomes: Versatile microcapsules in perspective. Colloids and Surfaces A: Physicochemical and Engineering Aspects 2009; 343:43-49.

[101] Hong M, Zhu S, Jiang Y, Tang G & Pei Y. Efficient tumor targeting of hydroxycamptothecin loaded PEGylated niosomes modified with transferrin. Journal of Controlled Release 2009; 133:96-102.

[102] Elnaggar YSR, El-Massik MA & Abdallah OY. Self-nanoemulsifying drug delivery systems of tamoxifen citrate: Design and optimization. International Journal of Pharmaceutics 2009; 380(1-2):133-141.

[103] Kim JC, Song ME, Kim MJ, Lee EJ, Park SK, Rang MJ, Ahn HJ. Preparation and characterization of triclosan-containing vesicles. Colloids and Surfaces B 2002; 26 235–241.

[104] Pardeike J, Hommoss A, Müller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. International Journal of Pharmaceutics 2009; 366 170–184.

[105] Cevc G. Lipid vesicles and other colloids as drug carriers on the skin, Advanced Drug Delivery Reviews 2004; 56 675– 711.

[106] Maestrelli F, Garcia-Fuentes M, Mura P, Alonso MJ. A new drug nanocarrier consisting of chitosan and hydoxypropylcyclodextrin, European Journal of Pharmaceuitics and Biopharmaceutics 2006; 63 79–86.

[107] Alvarez-Román R, Naik A, Kalia YN, Fessi H, Guy RH. Visualization of skin penetration using confocal laser scanning microscopy. European Journal of Pharmaceutics and Biopharmaceutics 2004; 58 301–316.

[108] Ogiso T, Shiraki T, Okajima K, Tanino TT, Iwaki MM. and Wada TT. Transfollicular drug delivery: penetration of drugs through human scalp skin and comparison of penetration between scalp and abdominal skins in vitro. Journal of Drug Targeting 2002; 10:369–78.

[109] Jacobi U, Toll R, Sterry W, Lademann J. Follicles play a role as penetration pathways in in vitro studies on porcine skin?. An optical study. Laser Physics Letters 2005; 15: 1594–98.

[110] Wilson T., (1990). Confocal Microscopy. Academic Press, London.

[111] Gerritsen HC, and De Grauw CJ. Imaging of optically thick specimen using two-photon excitation microscopy. Microscopy Research andTechnique 1999; 47, 206–209.

[112] Denk W, Delaney KR, Gelperin A, Kleinfeld D, Strowbridge BW, Tank DW, and Yuste R. Anatomical and functional imaging of neurons using 2-photon laser scanning microscopy. Journal of Neuroscience Methods 1994; 54, 151–162.

[113] Svoboda K, Denk W, Kleinfeld D, and Tank DW. In vivo dendritic calcium dynamics in neocortical pyramidal neurons. Nature 1997; 385, 161–165.

[114] Xu C, and Webb WW. Nonlinear and two-photon induced fluorescence. In:Topics in Fluorescence Spectroscopy. New York, USA: J. R. Lakowicz, editor, 1997.

[115] Denk WJ, Strickler JH, and Webb WW. Two-photon laser scanning fluorescence microscopy. Science 1990; 248, 73-76.

[116] Tsai TH, Jee SH, Dong CY, Lin SJ. Multiphoton microscopy in dermatological imaging. Journal Dermatological Science 2009; 56, 1-8.

[117] Paoli J, Smedh M, Ericson MB. Multiphoton laser scanning microscopy—a novel diagnostic method for superficial skin cancers. Seminars in Cutaneous Medicine and Surgery 2009; 28, 190-195.

[118] Chen T., Langer R., Weaver J. C. (1998) Skin electroporation causes molecular transport across the stratum corneum through localized transport regions. Journal of Investigative Dermatology Symposium Proceedings 1998; 3(2):159-65.

[119] Domínguez- Delgado CL. Estudio sobre el transporte a través de piel *in vitro* de triclosán, formulado en una dispersión de nanopartículas poliméricas, como alternativa para el tratamiento del acné. Efecto del laureato de sacarosa como promotor de absorción. Informe de investigación de Maestría. Posgrado en Ciencias Químicas, UNAM, 2008.

[120] Thiboutot D, Zaenglein A, Weiss J, Webster G, Calvarese B, Chen D. An aqueous gel fixed combination of clindamycin phosphate 1.2% and benzoyl peroxide 2.5% for the once-daily treatment of moderate to severe acne vulgaris: assessment of efficacy and safety in 2813 patients. Journal of American Academy of Dermatology 2008; 59: 792-800.

[121] Chien YW. (1987). Transdermal therapeutic systems. In: Controlled Drug Delivery Fundamentals and Applications. Robinson J. R., Lee V. H. L, editors. New York, USA: Marcel Dekker, Inc. p. 523-549.

[122] Schroeder U, Sommerfeld P, Ulrich S, Sabel BA. Nanoparticle technology for delivery of drugs across the blood–brain barrier. Journal of Pharmaceutical Science 1998; 87, 1305–1307.

[123] Raghuvanshi RS, Katare YK, Lalwani K, Ali MM, Singh O and Panda AK. Improved immune response from biodegradable polymer particles entrapping tetanus toxoid by use of different immunization protocol and adjuvants. International Journal of Pharmaceutics 2002; 245: 109–121.

[124] Leroux JC, Allemann E, De Jaeghere F, Doelker E, Gurny R. Biodegradable nanoparticles—from sustained release formulations to improved site specific drug delivery. Journal of Controlled Release 1996; 39, 339.

[125] Brannon-Peppas L, Blanchette JO. Nanoparticle and targeted systems for cancer therapy, Advanced Drug Delivery Reviews 2004; 56: 1649–1659.

[126] Haag R. Supramolecular drug-delivery systems based on polymeric core-shell architectures. Angewandte Chemie International Edition 2004; 43:278-282.

[127] Radowski MR, Shukla A, von Berlepsch H, Bottcher C, Pickaert G, Rehage H, Haag R. Supramolecular aggregates of dendritic multishell architectures as universal nanocarriers. Angewandte Chemie International Edition 2007; 46:1265-1269.

[128] Tinkle SS, Antonini JM, Rich BA, Roberts JR, Salmen R, DePree K, Adkins EJ. Skin as a route of exposure and sensitization in chronic beryllium disease. Environmental Health Perspectives 2003; 111:1202-1208.

[129] Kim S, Lim YT, Soltesz EG, De Grand AM, Lee J, Nakayama A. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. Nature Biotechnology 2004; 22:93-97.

[130] Symon Z, Peyser A, Tzemach D, Lyass O, Sucher E, Shezen E, Gabizon A. Selective delivery of doxorubicin to patients with breast carcinoma metastases by stealth liposomes. Cancer 1999; 86:72-78.

[131] Gonçalves A, Braud AC, Viret F, Genre D, Gravis G, Tarpin C. Phase I study of pegylated liposomal doxorubicin (Caelyx) in combination with carboplatin in patients with advanced solid tumors. Anticancer Research 2003; 23:3543-3548.

[132] Seiden M. V, Muggia F, Astrow A, Matulonis U, Campos S, Roche M. A phase II study of liposomal lurtotecan (OSI-211) in patients with topotecan resistant ovarian cancer. Gynecologic Oncology 2004; 93:229-232.

[133] El Maghraby GMM, Williams AC, Barry BW. Can drug-bearing liposomes penetrate intact skin? Journal of Pharmacy and Pharmacology 2006; 58:415-429.

[134] Weiner N, Williams N, Birch G, Ramachandran C, Shipman C, Flynn G. Topical delivery of liposomally encapsulated interferon evaluated in a cutaneous herpes guinea pig model. Antimicrobial Agents and Chemotherapy 1989; 33:1217-1221.

[135] Planas ME, Gonzalez P, Rodriguez L, Sanchez S, Cevc G. Noninvasive percutaneous induction of topical analgesia by a new type of drug carrier, and prolongation of local pain insensitivity by anesthetic liposomes. Anesthesia and Analgesia 1992; 75:615-621.

[136] Sentjurc M, Gabrijelcic V. Transport of liposome-entrapped molecules into the skin as studied by electron paramagnetic resonance imaging methods. In: Non-Medical Application of liposomes. Lasic B, editor. New York, USA; CRC Press.1995, pp 91-114.

[137] Cevc G., Gebauer D., Stieber J., Schatzlein A., Blume G., (1998) Ultraflexible vesicles, Transfersomes, have an extremely low pore penetration resistance and transport therapeutic amounts of insulin across the intact mammalian skin. Biochimica et Biophysica Acta 1998; 1368:201-215.

[138] Paul A, Cevc G, Bachhawat BK. Transdermal immunisation with an integral membrane component, gap junction protein, by means of ultradeformable drug carriers, transfersomes. Vaccine 1998; 16:188-195.

[139] Van den Bergh BA, Bouwstra JA, Junginger HE, Wertz PW. Elasticity of vesicles affects hairless mouse skin structure and permeability. Journal of Controlled Release 1999; 62:367-379.

[140] Guo J, Ping Q, Sun G, Jiao C. Lecithin vesicular carriers for transdermal delivery of cyclosporin A. International Journal of Pharmaceutics 2000; 194:201-207.

[141] Guo J, Ping Q, Zhang L. Transdermal delivery of insulin in mice by using lecithin vesicles as a carrier. Drug Delivery 2000; 7:113-116.

[142] Vrhovnik K, Kristl J, Sentjurc M, Smid-Korbar J. Influence of liposome bilayer fluidity on the transport of encapsulated substances into the skin, studied by EPR. Pharmaceutical Research 1998; 15:525-530.

[143] Egbaria K, Ramachandran C, Weiner N. Topical application of liposomally entrapped cyclosporin evaluated by *in vitro* diffusion studies with human skin. Skin Pharmacology 1991; 4:21-28.

[144] Cevc G, Schatzlein A, Richardsen H. Ultradeformable lipid vesicles can penetrate the skin and other semi-permeable barriers unfragmented. Evidence from double label CLSM experiments and direct size measurements. Biochimica et Biophysica Acta 2002; 1564:21-30.

[145] Honeywell-Nguyen PL, de Graaff A, Junginger HE, Bouwstra JA. Interaction between elastic and rigid vesicles with human skin *in vivo*. Proceedings International Symposium of Controlled Release Bioactive Materials 2000; 27:237-238.

[146] Esfand R, Tomalia DA. Poly(amidoamine) (PAMAM) dendrimer: from biomimicry to drug delivery and biomedical applications. Drug Discovery Today 2001; 6:427-436.

[147] D'Emanuele A, Attwood D. Dendrimerdrug interactions. Advanced Drug Delivery Reviews 2005; 57:2147-2162.

[148] Parekh HS. The Advance of Dendrimers - A Versatile Targeting Platform for Gene/Drug Delivery. Current Pharmaceutical Design 2007; 13:2837-2850.

[149] Niederhafner P, Šebestík J, Ježek J. Peptide dendrimers. Journal of Peptide Science 2005; 11:757-788.

[150] Cloninger MJ. Biological applications of dendrimers. Current Opinion in Chemical Biology 2002; 6:742-748.

[151] Medintz IL, Uyeda HT, Goldman ER, Mattoussi H. Quantum dot bioconjugates for imaging, labeling and sensing. Nature Materials 2005; 4:435-446.

[152] Caruthers SD, Wickline SA, Lanza GM. Nanotechnological applications in medicine. Current Opinion in Biotechnology 2007; 18:26-30.

[153] Arshady R. (1999).Microspheres, microcapsules, & liposomes. London: Citus books.

[154] Lademann J, Richter H, Teichmann A, Otberg N, Blume-Peytavi U, Luengo J, Weiss B, Schaefer UF, Lehr CM, Wepf R, Sterry W. Nanoparticles-an efficient carrier for drug delivery into the hair follicles. European Journal of Pharmaceutics and Biopharmaceutics 2007; 66:159–64.

[155] Bolzinger MA, Briançon S, Pelletier J, Chevalier Y. Penetration of drugs through skin, a complex rate-controlling membrane. Current Opinion in Colloid and Interface Science 2012; doi:10.1016/j.cocis.2012.02.001

[156] Müller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Advance Drug Delivery Reviews 2002; 54:S131-S155.

[157] Mei Z, Chen H, Weng T, Yang Y, Yang X. Solid lipid nanoparticle and microemulsion for topical delivery of triptolide. European Journal of Pharmaceutics and Biopharmaceutics 2003; 56:189-196.

[158] Liu W., Hu M., Liu W., Xue C., Xu H., Yang X. Investigation of the carbopol gel of solid lipid nanoparticles for the transdermal iontophoretic delivery of triamcinolone acetonide acetate. International Journal of Pharmaceutics 2008; 364:135-141.

[159] Ugazio E, Cavalli R, Gasco MR. Incorporation of cyclosporin A in solid lipid nanoparticles (SLN). International Journal of Pharmaceutics 2002; 241:341-344.

[160] Aditya NP, Patankar S, Madhusudhan B, Murthy RSR, Souto EB. Arthemeter-loaded lipid nanoparticles produced by modified thin-film hydration: Pharmacokinetics, toxicological and *in vivo* anti-malarial activity. European Journal of Pharmaceutical Science 2010; 40:448-455.

[161] Sanna V, Caria G, Mariani A. Effect of lipid nanoparticles containing fatty alcohols having different chain length on the ex vivo skin permeability of Econazole nitrate. Powder Technology 2010; 201:32-36.

[162] Joshi M, Patravale V. Nanostructured lipid carrier (NLC) based gel of celecoxib. International Journal of Pharmaceutics 2008; 346:124-132.

[163] Pardeike J, Müller RH. Coenzyme Q10 loaded NLCs: preparation, occlusion properties and penetration enhancement *(Cutanova Cream NanoRepair Q10).* Pharmaceutical Technology Europe 2007; 19: 46–49.

[164] Teeranachaideekul V, Souto EB, Junyaprasert V B, Müller RH. Cetyl palmitate-based NLC for topical delivery of Coenzyme Q10-Development, physicochemical characterization and *in vitro* release studies. European Journal of Pharmaceutical Science 2007; 67:141-148.

[165] Dingler A. Feste Lipid-Nanopartikel als kolloidale Wirstoffträgersysteme zur dermalen Applikation. Institut für Pharmazie. Freie Universität, Berlin, 1998.

[166] Puglia C, Filosa R, Peduto A, de Caprariis P, Rizza L, Bonina F, Blasi P. Evaluation of alternative strategies to optimize ketorolac transdermal delivery. AAPS Pharmaceutical Science Technology 2006; 7, 1–9 (Article 64).

[167] Teeranachaideekul V, Mülle, RH, Junyaprasert VB. Encapsulation of ascorbyl palmitate in nanostructured lipid carriers (NLC)-effects of formulation parameters on physicochemical stability. International Journal of Pharmaceutics 2007; 340, 198–206.

[168] Jenning V.Solid Lipid Nanoparticles (SLN) as a Carrier System for the Dermal Application of Retinol. Free University of Berlin, Berlin, 1999.

[169] Jenning V, Gohla SH. Encapsulation of retinoids in solid lipid nanoparticles (SLN). Journal of Microencapsulation 2001; 18, 149–158.

[170] Jee JP, Lim SJ, Park JS, Kim CK. Stabilization of all-trans retinol by loading lipophilic antioxidants in solid lipid nanoparticles. European Journal of Pharmaceutics and Biopharmaceutics 2006; 63, 134–139.

[171] Tsujimoto H and Hara K. Application 21-Development of functional skincare cosmetics using biodegradable PLGA nanospheres. Nanoparticle Technology Handbook (Second Edition), 2012, pp 501-506.

[172] Patzelt A, Richter H, Knorr F, Schäfer U, Lehr CM, Dähne L, Sterry W, Lademann J. Selective folicular targeting by modification of the particle sizes. Journal of Controlled Release 2011; 150:45–8.

[173] Shim J, Seok KH, Park W, Han S, Kim J, Chang I. Transdermal delivery of mixnoxidil with block copolymer nanoparticles. Journal of Controlled Release 2004; 97:477-484.

[174] Cetin ED, Savk E, Uslu M, Eskin M, Karul A. Investigation of the inflammatory mechanisms in alopecia areata. The American Journal of Dermatopathology 2009; 31: 53-60.

[175] Zaenglein AL, Graber EM, Thiboutot DM, Strauss JS, Wolff K, Goldsmith LA, Katz SI, Gilchrest B, Paller AS, Leffell D. J., editors., Chapter 78. Acne vulgaris and acneiform eruptions. 2009. Fitzpatrick's Dermatology in General Medicine. 7e: http://www.accessmedicine.com/content.aspx?aID=2963025

[176] Gollnick H, Cunliffe W, Berson D, Dreno B, Finlay A, Leyden JJ, Shalita AR, Thiboutot D. Management of acne: a report from the global alliance to improve outcomes in acne. Journal of the American Academy of Dermatology 2003; 49, S1-S37.

[177] Bojar RA, Holland KT. Acne and Propionibacterium acnes. Clinical Dermatology 2004; 22, 375–9.

[178] Ourique AF, Pohlmann AR, Guterres SS, Beck RCR. Tretinoin-loaded nanocapsules: Preparation, physicochemical characterization, and photostability study. International Journal of Pharmaceutics 2008; 352: 1–4.

[179] Lee P, Peng S, Su C, Mi F, Chen H, Wei M, Lin HJ, Sung HW. The use of biodegradable polymeric nanoparticles in combination with a low-pressure gene gun for transdermal DNA delivery. Biomaterials 2008; 29:742-751.

[180] Santander-Ortega MJ, Stauner T, Loretz B, Ortega-Vinuesa JL, Bastos-González D, Wenz G, Schaefer UF, Lehr CM. Nanoparticles made from novel starch derivatives for transdermal drug delivery. Journal of Controlled Release 2010;141:85-92.

[181] Thote AJ, Gupta RB. Formation of nanoparticles of a hydrophilic drug using supercritical carbon dioxide and microencapsulation for sustained release. Nanomedicine:Nanotechnology 2005; 1:85-90.

[182] McCarron PA, Hall M. Incorporation of novel 1-alkylcarbonyloxymethyl prodrugs of 5-fluorouracil into poly(lactide-co-glycolide) nanoparticles. International Journal of Pharmaceutics 2008; 348:115-124.

[183] Lboutounne H, Chaulet J, Ploton C, Falson F, Pirot F. Sustained ex vivo skin antiseptic activity of chlorhexidine in poly(ε-caprolactone) nanocapsule encapsulated form and as a digluconate. Journal of Controlled Release 2002; 82:319-334.

[184] Fuchs J, Zollner TM, Kaufmann R, Podda M. Redox-modulated pathways in inflammatory skin diseases. Free Radical Bioogy and Medicine 2001; 30: 337–53.

[185] Sanfilippo AM, Barrio V, Kulp-Shorten C, Callen JP. Common pediatric and adolescent skin conditions. Journal of Pediatric and Adolescent Gynecology 2003; 16: 269-83.

[186] Schäfer-Korting M, Mehnert W, Korting HC. Lipid nanoparticles for improved topical application of drugs for skin diseases. Advanced Drug Delivery Reviews 2007; 59, 427–443.

[187] Layton AM. Acne vulgaris and similar eruptions. Medicine 2005; 33: 44-48.

[188] Zouboulis ChC. Sebaceous glands, acne and related disorders: basic and clinical research, clinical entities and treatment. Journal of Investigative Dermatology 1997; 108: 371–98.

[189] Santos MC, Mehnert W, Schaller M, Korting HC, Gysler A, Haberland A, Schafer-Korting M. Drug targeting by solid lipid nanoparticles for dermal use. Journal of Drug Targeting 2002; 10, 489–495.

[190] Shah KA, Date AA, Joshi MD, Patravale VB. Solid lipid nanoparticles (SLN) of tretinoin: potential in topical delivery. International Journal of Pharmaceutics 2007; 345, 163–171.

[191] Cevc G, Gebauer D, Stieber J, Schatzlein A, Blume G.Ultraflexible vesicles, Transfersomes, have an extremely low pore penetration resistance and transport therapeutic amounts of insulin across the intact mammalian skin. Biochimica et Biophysica Acta 1998; 1368:201-215.

[192] Wang Z, Itoh Z, Hosaka Y, Kobayashi I, Nakano Y, Maeda I, Umeda F, Yamakawa J, Kawase M, Yag K. Novel Transdermal Drug Delivery System with Polyhydroxyalkanoate and Starburst Polyamidoamine Dendrimer. Journal of Bioscience and Bioengineering 2003; 95:541-543.

[193] Chauhan AS, Sridevi S, Chalasani KB, Jain AK, Jain SK, Jain NK, Diwan PV. Dendrimer-mediated transdermal delivery: Enhanced bioavailability of indomethacin. Journal of Controlled Release 2003; 90:335-343.

[194] Yiyun C, Na M, Tongwen X, Rongqiang F, Xueyuan W, Xiaomin W, Longping W. Transdermal delivery of nonsteroidal anti-inflammatory drugs mediated by polyamidoamine (PAMAM) dendrimer. Journal of Pharmaceutical Sciences 2007; 96:595-602.

[195] Venuganti VVK, Perumal OP. Effect of poly(amidoamine) (PAMAM) dendrimer on skin permeation of 5-fluorouracil. International Journal of Pharmaceutics 2008; 361:230-238.

[196] Cornwell PA, Barry BW. The routes of penetration of ions and 5-fluorouracil across human skin and the mechanisms of action of terpene skin penetration enhancers. International Journal of Pharmaceutics 1993; 94:189-194.

[197] Tsuji T, Sugai T. Topically administered fluorouracil in psoriasis. Archives in Dermatology 1975;105:208-212.

[198] Goette DK. Topical chemotherapy with 5-fluorouracil. A review. Journal of American Academy of Dermatology 1981; 4:633-649.

[199] Beall HD, Sloan KB. Topical delivery of 5-fluorouracil (5-FU) by 1, 3-bisalkylcarbonyl-5-FU prodrugs. International Journal of Pharmaceutics 2002; 231:43-49.

[200] Gao S, Singh J. Effect of oleic acid/ethanol and oleic acid/propylene glycol on the *in vitro* percutaneous absorption of 5-fluorouracil and tamoxifen and the macroscopic barrier property of porcine epidermis. International Journal of Pharmaceutics 1998; 165:45-55.

[201] Meidan VM, Walmsley AD, Docker MF, Irwin WJ. Ultrasound-enhanced diffusion into coupling gel during phonophoresis of 5-fluorouracil. International Journal of Pharmaceutics1999; 185:205-213.

[202] Merino V, Lopez A, Kalia YN, Guy RH. Electrorepulsion versus electroosmosis: effect of pH on the iontophoretic flux of 5-fluorouracil. Pharmaceutical Research 1999; 16:758-761.

[203] Lee WR., Shen SC, Wang KH, Hu CH, Fang JY. The effect of laser treatment on skin to enhance and control transdermal delivery of 5-fluorouracil. Journal of Pharmaceutical Science 2002; 91:1613-1626.

[204] Kuo F, Subramanian B, Kotyla T, Wilson TA, Yoganathan S, Nicolosi RJ. Nanoemulsions of an anti-oxidant synergy formulation containing gamma tocopherol have enhanced bioavailability and anti-inflammatory properties. International Journal of Pharmaceutics 2008; 363:206-213.

[205] Shakeel F, Ramadan W. Transdermal delivery of anticancer drug caffeine from water-in-oil nanoemulsions. Colloid Surface B 2010; 75:356-362.

[206] Wu H, Ramachandran C, Bielinska AU, Kingzett K, Sun R, Weiner ND, Roessler BJ. Topical transfection using plasmid DNA in a water-in-oil nanoemulsion. International Journal of Pharmaceutics 2001; 221:23-34.

[207] Subramanian B, Kuo F, Ada E, Kotyla T, Wilson T, Yoganathan S, Nicolosi R. Enhancement of anti-inflammatory property of aspirin in mice by a nano-emulsion preparation. International Immunopharmacology 2008; 8:1533-1539.

[208] Mou D, Chen H, Du D, Mao C, Wan J, Xu H, Yang J. Hydrogel-thickened nanoemulsion system for topical delivery of lipophilic drugs. International Journal of Pharmaceutics 2008; 353:270-276.

[209] Wu H., Ramachandran C, Weiner ND, Roessler BJ. Topical transport of hydrophilic compounds using water-in-oil nanoemulsions. International Journal of Pharmaceutics 2001; 220:63-75.

[210] Alves MP, Scarrone AL, Santos M, Pohlmann AR, Guterres SS. Human skin penetration and distribution of nimesulide from hydrophilic gels containing nanocarriers. International Journal of Pharmaceutics 2007; 341:215-220.