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Applications of nanoparticles in topical drug delivery and in cosmetics

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Abstract

The delivery of drugs and active agents to the skin by formulations containing nanoparticles is a topic of considerable current interest. A number of studies have shown important advantages of these nanostructure-based delivery systems over conventional formulations. This review describes the composition, preparation, and characteristics of a wide range of novel vectors, including nanoemulsions, liposomes, transfersomes, solid lipid nanoparticles, polymeric nanoparticles, ethosomes and niosomes.

Keywords: Nanoparticles, skin, stratum corneum, cosmetics, pharmaceuticals

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1 Introduction

The skin is the largest organ in the human body by weight, contributing about 10% of total weight, and covering an average area of 1.7 m². It regulates water and heat loss, and prevents the invasion of noxious chemicals and microorganisms. Because skin is an easily accessible organ, its potential as an alternative route for administering drugs for both systemic and local effect has attracted considerable interest [1]. Equally, a large segment of the cosmetic industry is focused on the delivery of “actives” to and into the skin. However, molecules do not easily penetrate the skin because of its excellent barrier function. As a result, various nano-carriers have been developed in an attempt to reversibly modulate the skin barrier and/or to provide novel delivery systems for the active of interest. These particulate carriers include nanoemulsions, liposomes, transfersomes, solid lipid nanoparticles, polymeric nanoparticles, ethosomes and niosomes. A schematic representation of their structures is shown in Figure 1.

2 Skin

2.1 The structure of the skin

The skin consists of the epidermis and the dermis, which sits on a layer of subcutaneous fat.

The epidermis contains four histologically distinct layers, from the innermost stratum basale via the stratum spinosum and stratum granulosum (SG) to the superficial stratum corneum (SC). The SC has been represented as a “brick and mortar” structure [2] in which the corneocytes are embedded in an intercellular lipid matrix. The corneocytes comprise insoluble keratins enveloped by cross-linked proteins, and are arranged in parallel, overlapping, multicellular stacks perpendicular to the skin surface [1]. The inter-corneocyte space is filled with lipids, usually present in the crystalline phase [3]. Most SC lipids are synthesized in the viable epidermis during differentiation [4], they are released into the intercellular spaces at the SG-SC interface from lamellar bodies. The major SC lipids are ceramides, fatty acids and cholesterol. Eight classes of ceramides have been identified. The lipids are arranged in multiple bilayers with a periodicity of about 13 nm. Unlike almost all other membranes in the body, the SC does not contain phospholipid [2]. This “brick and mortar” structure is now accepted as the location of the skin’s excellent permeability barrier, and the SC is the rate-limiting barrier to the transcutaneous penetration and absorption of most chemicals following topical administration [5].

There are three possible pathways of molecular penetration across the SC: (i) intercellular via the lipids between the corneocytes; (ii) transcellular crossing through the corneocytes and the surrounding lipids; (iii) appendageal via follicles and sweat ducts (Figure 2) [6]. The principal route is generally believed to be intercellular. The principal route is generally believed to be intercellular, although the appendageal route, in particular that encompassing the hair follicle and associated sebaceous gland, is also important in certain circumstances [7-12] and may offer an opportunity for drug targeting in the treatment of hair loss or acne, for example [13-16]. Some permeation enhancers (e.g., oleic acid) and vesicular carriers are thought to disorder the SC lipids and facilitate transport across the skin [17-19].

Underlying the SC is the viable epidermis, the thickness of which is typically ~100 μm ,

ranging from as little as 50 μm to around 800 μm on the load-bearing palms and soles of the feet [1]. The principal cells of the viable epidermis are keratinocytes, but there are also melanocytes, Langerhans cells, migrant macrophages and lymphocytes [20]. However, there are no blood vessels in the epidermis.

The dermis is typically 3–5 mm thick and is the major component of human skin. It is rich in blood vessels, lymphatic vessels and nerve endings. The skin appendages, which include hair follicles, sebaceous glands and sweat glands, also originate in the dermis. This layer resembles an aqueous gel and is a minimal barrier to drug transport. As mentioned above, the hair follicles and associated sebaceous glands are considered to play a role with respect to transport across the skin; given that sebum consists mostly of neutral, non-polar lipids, it may be anticipated that this route favours more lipophilic permeants.

The subcutaneous fat layer bridges between the dermis to the underlying tissue, and plays a negligible role in the percutaneous absorption of topically applied substances.

2.2 Techniques to evaluate nanoparticle disposition on and within the skin

To evaluate nanoparticle penetration disposition on/ and/or within the skin, several methods have been employed, including Franz-type diffusion cell experiments, differential tape-stripping of the stratum corneum [21,22], laser scanning confocal microscopy (LSCM) [23-27], multi-photon fluorescence microscopy (MFM) [28], and transmission electron microscopy (TEM) [29]. These techniques have permitted qualitative and semi-quantitative deductions about the extent and mechanisms of nanoparticle uptake into the stratum corneum to be deduced.

3 Nanoemulsions

3.1 Composition and preparation

Nanoemulsions are stable dispersions with mean droplet diameters of a few hundred nanometers, and are sometimes called sub-micron or mini-emulsions. These systems are composed of oil, water, and one or more surface-active agents, and may be oil-in-water (o/w) or water-in-oil (w/o) dispersions [30]. In some circumstances, nanoemulsions may be formed using phospholipids as one of the surface-active constituents; if the level of lipid is high, the concurrent formulation of liposomes is possible [31]. The aqueous phase may contain hydrophilic, pharmaceutical or cosmetic active ingredients and preservatives, while the oil phase is typically composed of mineral oil, silicone oil, vegetable oil, esters of fatty acids, and/or lipophilic active ingredients. Surfactants, such as disodium stearyl glutamate, sucrose alkyl ester, sorbitan alkyl ester and dimethicone copolyol, are added to the formulation to allow formation of a stable dispersion and guarantee an appropriate shelf life of the product.

Both o/w and w/o nanoemulsions can be used in pharmaceutical preparations for topical administration. In the former case, the common oil core constituents are triglycerides, propylene glycol mono caprylic ester, cholesteryl esters and cholesterol [32]. Most nanoemulsions in cosmetics are oil-in-water and contain 10-20% oil stabilized with 0.5-2% emulsifying agent. Popular oils are triglycerides, silicones, isopropyl myristate, isocetyl isostearate and isododecane. As well as conventional surfactants, polymeric emulsifiers, such as carbomers and hydroxypropyl methylcellulose (HPMC), are also being used to produce stable products with a pleasant appearance [33, 34]. Carbomers are crosslinked polyacrylic acid polymers; and represent the most widely used thickening agents in skincare products [35]. Once introduced into a nanoemulsion, irregular structures on the order of a micron in size are observed (Figure 3), in proportion to the amount of carbomer employed. Carbomers can form a thick protective gel layer around each oil droplet and increase the viscosity of the external phase. Following contact with the skin, electrolytes from the skin surface cause the protective gel layer to deswell instantly. The oil phase is released and a thin film is deposited on the skin. This mechanism permits the convenient formulation of sun-care products which are

ultimately waterproof despite their predominantly hydrophilic properties prior to application. The emulsification mechanism of HPMC is similar to that of carbomer, although the former is less sensitive to the presence of electrolytes. It is believed that the mechanical stress, imparted on application of these emulsions, causes a partial breakdown of their structures such that a thin film of oil spreads over the skin surface, reducing its wettability. After the water has evaporated, a flexible film remains consisting of oil droplets embedded into the polymer matrix [33].

Nanoemulsions are easily produced in large quantities by mixing a water-immiscible oil phase into an aqueous phase using a high-stress, mechanical extrusion process [36, 37].

3.2 Applications of nanoemulsions in pharmaceuticals

An o/w nanoemulsion containing 10% (m/m) oil (propylene glycol mono-caprylic ester and glycerol triacetate), 50% (m/m) surfactant (diethylene glycol monoethyl ether and Tween-80) and 40% (m/m) water has been suggested as a vehicle for the improved transdermal delivery of celecoxib [38]. *In vitro* skin permeation studies showed enhanced percutaneous uptake of the drug from the nanoemulsion relative to a simple gel [38]. *In vivo*, inhibition of carrageenan-induced paw edema in rats was observed when celecoxib nanoemulsion was used. The ability of nanoemulsion formulations to enhance topical drug delivery has also been shown for ketoprofen [39]. It was claimed that the drug permeation rate could be manipulated by changing the relative amounts of oil, surfactants and co-surfactants. Similarly, another study with an aceclofenac nanoemulsion showed improved permeation of the drug into rat abdominal skin, and significantly increased anti-inflammatory effect on carrageenan-induced paw edema in rats *in vivo*, when compared with a gel formulation [40].

As well as acting as a drug carrier, nanoemulsions themselves have extensive antimicrobial activity against bacteria (e.g., *E. coli*, *Salmonella*, and *S. aureus*), viruses (e.g., HIV, Herpes simplex), fungi (e.g., *Candida*, Dermatophytes), protozoa and spores (e.g., anthrax) due to their ability to fuse with and lyse these different organisms. Fusion is primarily driven by the electrostatic attraction between the typically cationic charge of the emulsion and the anionic charge on the pathogen. There is one example of the antimicrobial effects of surfactant

nanoemulsions. A w/o nanoemulsion (X8W₆₀PC), containing oil (64%), detergents (9.7%), solvent (8%) and water (18.3%), at a low concentration of 1%, significantly reduced the number of colony forming units (CFU) of *Candida. albicans* by more than four logs within 15 minutes of treatment, and by six logs in a two-hour exposure. Because some of the ingredients of X8W₆₀PC are biocidal, the anti-fungal activity of the individual ingredients was also evaluated at concentrations equivalent to those in X8W₆₀PC. However, none of the constituents was as effective a fungicidal as the nanoemulsion (Figure 4), suggesting that the activity of X8W₆₀PC depends upon its nanoemulsion structure [41].

A special type of topical nanoemulsion has been developed for the instillation of an adrenergic β -blocking agent, adaprolol maleate, into the eye, to treat glaucoma without inducing systemic side-effects. However, the drug has an important clinical disadvantage of irritation to the eye, causing an immediate burning sensation and local discomfort. A nanoemulsion formulation, on the other hand, maintains the therapeutic efficacy of the drug while reducing the level of ocular irritation [32].

3.3 Applications of nanoemulsions in cosmetics

Nanoemulsions can be found in a wide variety of cosmetic products such as bath oils, body creams, anti-wrinkle and anti-aging preparations. Due to their small and uniform droplet size, nanoemulsions are transparent, fluid and pleasant to touch [42, 43]. In comparison to traditional emulsions, nanoemulsions have better spreading properties on the skin. These unique texture and rheological properties make them very valuable in cosmetic technology. Formulations containing nanoemulsions range from water-like fluids to semi-solid gels.

Numerous patents reflect the active development of nanoemulsion formulations. For example, nanoemulsions containing fluid, non-ionic, amphiphilic lipids, such as diglyceryl isostearate, sorbitan oleate, and α -butylglucoside caprate, were stable on storage between 0°C and 45°C [44], were able to contain significant amounts of fragrance, and promoted the penetration of “actives” into the surface layers of the skin. Nanoemulsions made with anionic, amphiphilic lipids of phosphoric acid fatty esters and oxyethylenated derivatives also retained transparency and good cosmetic properties even when large amounts of oil were added to the formulations [45]. Another o/w nanoemulsion based on one or more nonionic and/or anionic

amphiphilic lipids, and one or more water-soluble neutral polymers (e.g. poly-(ethylene oxide), polyvinyl alcohols; polyvinylcaprolactam), allowed the viscosity of the composition to be increased without influencing its transparency or increasing the level of the oil phase [46]. A stable and translucent nanoemulsion for cosmetic, dermatological and/or ophthalmological applications comprised a ternary surfactant system of ethoxylated fatty ester polymer, fatty acid ester of sorbitan and alkali metal salts of cetyl phosphate or palmitoyl sarcosinate, did not require gelling agents for stabilization [47], making it suitable for use on sensitive skin. Several other nanoemulsion technologies have been developed for diverse properties, including sun-protection, anti-wrinkling, anti-aging of the skin and other cosmetic targets [48-52].

3.4 Summary

Nanoemulsions consist of fine o/w or w/o dispersions. They are used in dermatology to improve drug delivery to and through the stratum corneum. In addition, nanoemulsions themselves are biocidal towards bacteria, viruses, fungi, protozoa and spores. In cosmetics, nanoemulsions have found use in many cosmetic products for their transparent visual aspect, hydrating power and good skin feel.

4 Liposomes

4.1 Composition and preparation

Liposomes are spherical vesicles consisting of one or more membrane-like phospholipid bilayers enclosing an aqueous core. Vesicle diameter ranges from fifty to several hundred nanometers. The principal lipid component of liposomes is typically phosphatidylcholine (PC) derived from egg or soybean lecithin [53]. Cholesterol is usually included in the composition to stabilize the structure thereby generating more rigid liposomes [54]. Depending on the processing conditions and the chemical composition, small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), large multilamellar vesicles (MLV) and multivesicular vesicles (MVV) may be formed with one or several concentric bilayers (Figure 5). Unlike emulsions, liposomes are thermodynamically stable lamellar structures which form spontaneously when lipid is brought into contact with an aqueous phase [55].

Liposomes can be prepared by a number of methods. The major techniques are lipid film hydration, emulsification, reverse phase evaporation, freeze-thaw processes, and solvent injection. Large liposomes form spontaneously when phospholipids are dispersed in water above their phase transition temperature. To prepare small vesicles, an appropriate technique, such as high-pressure homogenization, sonication, or extrusion, is required to reduce particle size. It should be stated that most of the methods for preparing liposomes have at least one of the following drawbacks: use of large quantities of solvent, need for special equipment, and low “active” encapsulation efficacy.

Due to their biphasic character, liposomes can act as carriers for lipophilic, amphiphilic and hydrophilic substances. The entrapped compound's solubility and partitioning characteristics will determine its location in the liposomal bilayer, its level of association with the liposome and its release rate. In general, lipophilic and amphiphilic substances, e.g., oil-soluble UV filters, are located in the lipid bilayer of the liposome. As such compounds are very poorly water-soluble, loss of entrapped drug on storage is minimal. Hydrophilic drugs are entrapped inside the aqueous core of liposomes, but may also be in the external water phase. The percentage of encapsulated hydrophilic drug depends on the liposome bilayer

composition and the preparation procedure.

4.2 Applications of liposomes in pharmaceuticals

The first report of the use of liposomes in topical drug delivery involved delivery of triamcinolone acetonide. It was claimed that the liposomal formulation significantly increased the concentration of steroid achieved in the epidermis and dermis [56]. In a further study, it was reported that application of triamcinolone acetonide-loaded liposomes resulted in ~5-fold more drug accumulation within the epidermis in comparison to a more conventional gel formulation [57]. A number of other studies have implied the efficiency of liposome formulations to deliver enhanced drug amounts to the upper skin layers. For example, a liposome formulation of betamethasone dipropionate out-performed a commercial conventional formulation containing a higher concentration of drug in a clinical trial considering the treatment of atopic eczema [58]. The liposome formulation was less efficient in treating psoriasis for which deeper penetration of the drug is needed. Econazole, topical antifungal drug, can be irritant to the skin when topically applied in conventional vehicles. The application of liposome formulation has been shown to be a good strategy to minimize this irritation and enhance patient compliance. In biodisposition studies with a econazole in a liposomal gel dosage form, an ~7-fold increase in drug concentration in the epidermis was achieved, relative to a control cream [59]. It was possible, therefore, to reduce the applied drug dose yet maintain an equivalent therapeutic efficacy, thereby minimizing skin irritation. Similar results have been obtained with minoxidil, a drug to combat hair loss, and several liposomal products have been shown to be more efficient in delivering the drug to hair follicles than conventional dosage forms [59, 60]. Likewise, the local anesthetic agents, tetracaine and lidocaine, showed enhanced activity over conventional dermatological preparations when liposomal products were used [61, 62].

Of course, the efficiency of topical drug delivery from liposomal vehicles depends on the physicochemical properties of drug involved [63]. For example, the release of progesterone from Intralipid® emulsion was substantial, whereas egg-phosphatidylcholine and dipalmitoylphosphatidylcholine liposomal formulations delivered only 1% of the drug “payload” over a similar period [64]. The release of progesterone from liposomes followed

zero-order kinetics, controlled by slow interfacial transport of the drug from the bilayer into the surrounding aqueous medium.

When caffeine (3% w/v) was delivered from (i) an aqueous solution, (ii) a PEG solution, (iii) an aqueous solution containing the enhancers, transcutool and oleic acid, (iv) a PEG-water solution with the same enhancers, and (v) a phosphatidylcholine/cholesterol liposomal (SV) formulation, the results in Figure 6 were obtained [65]. Notably, the enhancers were effective, and interestingly the vesicles significantly retarded the delivery of caffeine. This resulted, after 24 hours, in a much higher retention of the active in the epidermis following SV application. The same improved accumulation of drug in the skin has also been reported for a liposome formulation containing unfractionated heparin [66].

The exact manner in which topical liposomes interact with the SC, and the lipids therein, is not fully characterized, especially when this occurs in the presence of an organic solvent, such as ethanol. Enhanced mixing with the intercellular lipids of the SC and/or the sebaceous lipid on the surface and within the hair follicles seems likely and logical. Microscopic observations have confirmed the fusion of liposomes on the SC surface resulting in stacks of lamellae and other, irregular structures [67]. It must be also remembered that the amount of lipid in the SC intercellular spaces may be small, relative to that applied in the form of liposomes. A square centimeter of SC of thickness of 10 μm has a volume of 1 μl ; given the “brick-and-mortar” structure of the SC, ~20% may be assumed to be associated with intercellular (and surface) lipid, which is 0.2 μl of lipid. Taking lipid density as about 1 g/ml (1 mg/ μl), then the lipid content of 1 cm^2 of SC is approximately 0.2 mg, an amount comparable to or smaller than the levels of lipid typically used when assessing the impact of formulations. It is perhaps not surprising, therefore, that enhanced deposition of drug into the SC is found when these preparations are used.

4.3 Applications of liposomes in cosmetics

Liposomes are found in numerous products designed to deliver active cosmetic substances into the epidermis. The aim is to concentrate the active ingredients in the outermost skin layers. For example, liposome-encapsulated UV filters incorporated into aqueous-based sunscreens products have good substantivity on the skin surface, thereby preventing them

from being easily washed off. Liposomes themselves, when formulated into cosmetics, can replenish/augment the endogenous SC lipids, increase moisturization and reduce skin dryness [68].

There are many marketed liposomal cosmetics. Capture[®] was the first product incorporating liposomes and was introduced by C. Dior in 1986. It contains 5% thymus extract, 1% collagen and elastin peptides, and 0.1% hyaluronic acid in liposomes (100 nm diameter) made from soya lecithin [69]. Estée Lauder's "Advanced Night Repair Protective Recovery Complex[®]" contains a liposome delivery system which is claimed to neutralize and repair 90% the damage caused by free radicals generated by UV, pollutants and oxidants. The formulation contains hyaluronic acid and is an effective moisturizer too. L'Oréal has pioneered the development of nanosomes (i.e., very small liposomes) in an anti-wrinkle product, "Revitalift[®] Double Lifting", containing pro-retinol A [70]. Jafra Cosmetics International's "Royal Jelly Lift Concentrate[®]" includes liposomes and a complex mixture of amino acids, vitamins and minerals, to stimulate cell renewal and prevent wrinkles [71]. Overall, cosmetic preparations containing liposomes range from simple creams and gels to complex formulations containing various extracts, moisturizers, antibiotics, and recombinant proteins for wound or sunburn healing. The commercial products are available as anti-aging skin creams, sunscreens, long-lasting perfumes, hair conditioners and so on.

4.4 Summary

The use of liposomes for the topical delivery of drugs and cosmetic actives represents a huge area of activity. The lipids comprising the vesicles clearly mix with endogenous SC lipids and transfer their encapsulated "payload" into the skin, sometimes undermining barrier function, at others providing reinforcement and improving hydration. Retention of an active at the SC surface can also be achieved as a positive benefit (e.g., for a sunscreen). Many cosmetic products based on liposomes have reached the market.

5 Transfersomes

5.1 Composition and characteristics

Transfersomes[®] (IDEA AG), are highly deformable mixed lipid aggregates, regarded as “elastic liposomes”. They differ from liposomes because of the presence of so-called edge-activators [72], and comprise phospholipids as the main ingredient with 10-25% surfactant (e.g. sodium cholate) and 3-10% ethanol. The surfactants are the “edge activators”, which confer ultradeformability on the transfersomes [73]. The elasticity of the vesicle is correlated with the quantity and the structure of the incorporated surfactant [74]. In comparison with liposomes, it has been claimed that transfersomes are able to deliver their “payload” deeper into the skin [58, 75].

5.2 Interaction of transfersomes with the skin

The proposed driving force for the putative penetration of transfersomes across the skin is the water activity gradient between the relatively dehydrated skin surface and the aqueous viable epidermis [76]. Hence, when a transfersome formulation is applied on the skin under non-occlusive conditions, the evaporation of water from the vehicle drives the penetration of vesicles towards the viable epidermis to avoid their dehydration [77]. It has been reported that transfersomes penetrate into the SC via two different hydrophilic pathways [78, 79]: (i) An intercluster route via the “gorge” between corneocytes clusters (formed by 3 to 10 individual corneocytes) which has a uniform width ($\leq 4\text{-}6\ \mu\text{m}$) and depth ($\leq 3\text{-}5\ \mu\text{m}$) (Figure 7); this pathway has a relatively low penetration resistance and corresponds to $\leq 1\%$ of the total skin surface area. (ii) The intercorneocyte pathway travels between the individual corneocytes in the cell clusters, and occupies an area greater than 3% of the total skin surface area.

5.3 Applications of transfersomes in pharmaceuticals

The anti-inflammatory action of topical triamcinolone acetonide delivered from transfersomes was significantly greater than that induced by marketed products [80]. Moreover, the biological activity of the drug was maintained at doses at least one order of magnitude lower than that commonly used in commercial topical lotions or creams. As a

result, it may be anticipated that any systemic side effects would be greatly reduced.

It has also been reported that diclofenac associated with ultradeformable transfersomes is a good alternative for the combined oral and topical administration of the drug for rheumatoid disease [81]. Delivery from transfersomes sustained a prolonged therapeutic effect and resulted in a higher drug concentration in the skin than that from a commercial hydrogel.

The topical administration of oestradiol from transfersomes and from rigid liposomes has been compared [82], and the deformable vesicles were shown to be significantly superior. This enhanced delivery was maintained for different “edge activators” [83]. Similarly, phosphatidylcholine vesicles containing sodium cholate, Span-80 or oleic acid significantly enhanced oestradiol transport across human skin *in vitro* relative to control formulations in which the transfersome constituents were present, but had not been assembled into the ultradeformable particles [84]. Similarly, flexible liposomes containing cyclosporin A delivered more drug into the skin than conventional vesicles [85].

Finally, and remarkably, transfersomes have also been claimed as a technology for the non-invasive delivery of insulin across the skin [86, 87], although the ultimate practicality of the approach remains to be established.

5.4 Summary

Transfersomes have been claimed to enhance significantly the local and systemic delivery of a wide range of compounds. The osmotic gradient across nonoccluded skin has been proposed as the driving force for this improved delivery when ultradeformable vesicles are used. Initial applications are focused upon non-steroidal anti-inflammatory drugs with a site of action beneath the skin in the subcutaneous tissue.

6 Polymeric nanoparticles

6.1 Nanocapsules

6.1.1 Composition and preparation

Polymeric nanocapsules are submicron colloidal particles with a core surrounded by a polymeric shell. In general, the core of nanocapsules is an organic (oil) solvent. Some of the most widely used polymers for the nanocapsule shell are poly-(ϵ -caprolactone) (PCL) [88], poly-L-lactide (PLA) [89], poly-(glycolic acid) (PGA), poly-(lactide-co-glycolide) (PLGA) [90], poly-(butylcyanoacrylates) [91-94], poly-(ethylcyanoacrylates) [95], poly-(alkylene adipate) [96], polyvinyl acetate (PVA) [97], cellulose acetate phthalate [98], poly-(ϵ -caprolactone)-block-poly-(ethylene glycol) [99], poly-(methyl methacrylate) [93], and polystyrene.

Methods for preparing nanocapsules can be classified into two categories. The first involves in situ interfacial polymerization around a droplet [100]; the second requires interfacial nanodeposition of a preformed polymers [101]. In interfacial polymerization, either a monomer, or an amphiphilic polymer with a cross-linkable group, is used and polymerization is induced at the surface of a droplet. A w/o or o/w emulsion is usually formed first before the shell is polymerized at the interface of two phases [102, 103]. This technique allows the polymeric shell to follow the contour of the inner phase. The drawback is that the polymerization process may provoke side-reactions involving the drug or active ingredient and reduce thereby their ultimate availability from the formulation. In preparing nanocapsules from preformed polymers, the deposition of the polymer at the surface of an oil droplet can be achieved by mixing the organic phase containing the polymer with an aqueous phase containing a hydrophilic surfactant. As the nanometer-sized droplets of oil form, the polymer precipitates at the interface with the aqueous phase and the nanocapsules are stabilized by the surfactant [104]. This technique avoids some of drawbacks of the interfacial polymerization process, such as lack of control of molecular weight, the presence of residual monomer in the preparation, and the possibility of side-reactions.

6.1.2 Applications of nanocapsules

Nanocapsules have been proposed as topical formulation constituents for several active compounds, including diclofenac, lidocaine, caffeine, retinoids, vitamin E, beta-carotene, amino acids, plant extracts, fragrances, antioxidants and UV protectants. The nanocapsule core has the advantage of providing a high loading capacity, with a relatively low polymer content. Compared to liposomes, polymeric nanocapsules are more robust as the shell is a covalently linked structure.

6.1.2.1 Applications of nanocapsules in pharmaceuticals

The first polymeric particle system used for transdermal drug delivery comprised PLA microcapsules containing the contraceptive steroid, levonorgestrel [89]. Subsequently, indomethacin was encapsulated in poly-n-butylcyanoacrylate nanocapsules and improved the transdermal delivery of the drug compared with a conventional gel [94]. Nanoencapsulation of flufenamic acid using PLGA nanocapsules also resulted in significantly increased drug accumulation in the viable skin layers when assessed *in vitro* using different experimental models [90]. Delivery of chlorhexidine from PCL nanocapsules synthesized by interfacial polymerization has been assessed on eight bacteria strains. Sustained drug release was achieved and a prolonged *ex vivo* topical antimicrobial activity on porcine ear skin against *Staphylococcus epidermis* was reported [105]. The improved efficacy was explained by a more direct and sustained contact between the particles, bacteria, skin surface and hair follicles.

6.1.2.2 Applications of nanocapsules in cosmetics

Generally, nanocapsules are used in cosmetics to protect sensitive actives, reduce undesirable odours and avoid incompatibility between formulation ingredients. One of the first nanocapsule-based products was an anti-wrinkle cream encapsulating vitamin A; the particles acted as reservoirs, slowly releasing the active over time [106]. Recently, L'Oréal marketed two products, Primordiale Intense and Hydra Zen Serum, which use nanocapsules to encapsulate several active ingredients.

Nanocapsules have also been intensively investigated as sunscreen vehicles for octyl

methoxycinnamate (OMC), octyl salicylate and benzophenone-3. It is believed that the nanocapsules form a protective film on the skin surface and retard any penetration of the active sunscreen into the viable tissue. For example, PCL has been used to prepare OMC-loaded nanocapsules which were more effective in protecting against UVB radiation than a conventional gel [88]. OMC was also encapsulated in cellulose acetate phthalate nanocapsules and its accumulation in the SC was compared to that from a nanoemulsion [98]. In this case, the nanocapsules were less efficient in delivering OMC. The encapsulation of benzophenone-3 (oxybenzone) in a series of nanoparticles made from PVA-fatty acid (Figure 8) with different molecular weights has been reported [97]. The nanocapsules significantly decreased the transport of oxybenzone through porcine ear skin *in vitro* by more than an order of magnitude (Figure 9)

6.2 Nanospheres

Nanospheres comprise a homogeneous matrix of polymer in which the drug or active ingredient is dispersed throughout. Generally, methods used to prepare nanospheres can also be adapted to the manufacture of nanospheres [100], i.e., interfacial polymerization, emulsion polymerization and solvent evaporation [30].

For example, the controlled release of captopril from poly-butylcyanoacrylate nanoparticles has been achieved, but improved skin transport was not observed [92]. Somewhat improved delivery of minoxidil from poly-(caprolactone)-block-poly-(ethylene glycol) nanoparticles was reported, with a better result achieved from that of smaller diameter (40 versus 130 nm) [99]. Further, cyclosporine A loaded into chitosan nanospheres better maintained an effective drug concentration on the ocular surface (for up to 48 hours) while avoiding systemic exposure, rapid clearance, eye irritation and blurred vision [107].

6.3 Summary

Polymeric nanoparticles are unable to cross intact SC. Drugs incorporated into nanoparticles may be slowly released onto the skin surface and into the superficial skin strata. Nanoparticles incorporated in cosmetic preparations can protect unstable active ingredients, avoid incompatibility between different ingredients, and ensure that the absorption of

sunscreens, for example, is avoided by forming a film on the skin surface. However, the number of products on the market that are based on polymeric nanoparticles is limited. This is due to a number of factors including the cytotoxicity of some polymers, and problems of scaling up the complex preparation methods involved.

7 Solid lipid nanoparticles (SLN)

7.1 Composition and preparation

SLN are sub-micrometer in size [108, 109]. The lipids employed include triglycerides, partial glycerides, fatty acids, steroids and wax. Different emulsifiers have been utilized to stabilize SLN dispersions, including Poloxamer 188, Polysorbate 80, lecithin, polyglycerol methylglucose distearate, sodium cocoamphoacetate and saccharose fatty acid esters [110].

The two principal SLN preparation methods are high pressure homogenization and via microemulsion formation. The former is subdivided into hot and cold techniques [109, 111-114]. Hot homogenization is the most frequently used. In this method, the lipid melt is dispersed in a hot surfactant solution at the same temperature by high-speed stirring. The resulting pre-emulsion is passed through a high pressure homogenizer to produce a hot o/w nanoemulsion. The lipid recrystallizes to SLN as the hot nanoemulsion is cooled to room temperature. It is noteworthy that for lipids (e.g., glycerides) with a low melting point close to room temperature, the nanoemulsion needs to be cooled to even lower temperatures to initiate recrystallization. The hot homogenization method may be suitable for temperature-sensitive compounds, because the time of exposure to an elevated temperature is relatively short [109].

The cold homogenization technique is recommended for preparing SLN containing either highly temperature-sensitive compounds or hydrophilic compounds which can partition from the melted lipid phase to the water during a hot homogenization process [111]. In the cold homogenization technique, the melted lipid containing the drug is cooled, and then ground to microparticles which are subsequently dissolved in a cold surfactant solution to form a pre-emulsion. This is then homogenized into SLN at or below room temperature. As homogenization can cause an increase in temperature, the difference between the lipid melting point and the homogenization temperature should be large enough to avoid melting of lipid in the homogenizer.

When SLN are produced by the microemulsion technique, the melted lipid and the aqueous surfactant solution must be at the same temperature. Surfactants and co-surfactants used include lecithin, bile salts and alcohols such as butanol. The two phases are mixed in the

correct ratio to form a microemulsion, which is then dispersed in a cold aqueous medium (2-3°C) under gentle mechanical mixing, to ensure that small particles are formed by precipitation [115].

SLN can be also produced by a precipitation method [116]. The lipid is dissolved in an organic solvent and an aqueous phase is added provoking emulsification [117]. The solvent is then evaporated and the lipid precipitates forming nanoparticles. A disadvantage of this method is the use of organic solvent.

7.2 Applications of SLN in pharmaceuticals

SLN have been used to enhance the topical delivery of several drugs. For example, SLN containing tristearin glyceride, soybean lecithin and polyethylene glycol 400 stearate increased the transport of triptolide *in vitro* by 3 to 4-fold over that from a simple solution of the drug [118]. In eczema patients, SLN loaded with clobetasol propionate showed improved efficacy over a conventional cream [119]. Prednicarbate associated with SLN (again for eczema treatment) was better delivered to the viable epidermis, with less exposure to (and atrophy of) the underlying dermis [113, 120], suggesting a possible targeting effect. Antiandrogens for acne treatment have also been shown to have better efficacy when topically applied in SLN formulations [121]. Once again, drug was concentrated in the outer skin layer and deeper penetration to underlying tissues and to the systemic circulation would be expected to be minimal. It was also shown that the lipophilic fluorescent marker, Nile Red, was delivered from the SLN to the hair follicle infundibulum, once more implying an ability to target a specific skin structure.

7.3 Applications of SLN in cosmetics

After topical application, SLN can form an occlusive adhesive film on the skin surface [122-126]. For example, an SLN formulation containing tocopherol acetate was twice as occlusive as an emulsion with identical lipid content (Figure 10) [123]. Scanning electron microscopy was used to visualize the SLN on a single tape, and showed complete film formation without distinguishing individual particles (Figure 11).

SLN appear quite attractive as components for sunscreen products. Not only does the lipid

matrix at the skin surface retard the penetration of the active, molecular UV absorbers themselves (thereby reducing potential toxicity relative to conventional formulations) [111, 123, 127-130], the SLN on their own have a sun-protective effect by efficiently scattering the radiation falling on the skin (similar to titanium dioxide particles [131]). Cetyl palmitate SLN containing molecular sunscreens have been shown to have this synergistic photoprotection effect [111, 123, 128, 131].

Moreover, incorporation of chemically labile active ingredients (e.g., coenzyme Q10, retinol, and tocopherol) into SLN offers protection against decomposition [132-137] and, finally, it should be mentioned that the controlled release of an active ingredient is possible from SLN either to provide a burst release or a more sustained delivery profile [127]. This feature has been exploited for the controlled release of retinol and oxybenzone from SLN incorporated into creams and hydrogels [128, 135].

7.4 Summary

SLN have formed a wide range of applications and have useful properties for maintaining “actives” on the SC surface or within the upper skin layers. Their ability to form adhesive, occlusive films is particularly useful for sunscreen applications and for maintaining the stability of labile chemicals.

8 Ethosomes

8.1 Composition and characteristics

Ethosome are primarily composed of phospholipids, relatively high concentrations of ethanol, and water [138-141]. Their average diameter ranges from tens of nanometers to microns, and depends upon the relative amounts of phospholipids and ethanol [138]. Similar to liposomes, ethosomes can be unilamellar [142, 143] or multilamellar [138, 139, 144].

A number of methods have been used to prepare stable ethosomal formulations depending on the drug characteristics and on the drug delivery target [140, 141]. The manufacturing processes are easily scaled-up. The presence of ethanol allows for efficient entrapment of hydrophilic, lipophilic and amphiphilic molecules. This feature is illustrated by research in which three fluorescent probes of distinct physicochemical properties were encapsulated in ethosomes and liposomes, and their behaviour was then examined by laser scanning confocal microscopy (LSCM) [138]. The three dyes were the lipophilic Rhodamine Red, dihexadecanoyl glycerophosphoethanolamine (RR), the amphiphilic 4-(4-diethylamino) styryl-N-methylpyridinium iodide (D-289), and the hydrophilic calcein. Figure 12 shows that the lipophilic and amphiphilic drugs were clearly associated with the liposomal bilayer, while calcein was concentrated in the aqueous core. In contrast, all the dyes were found throughout the entire volume of the ethosomes at high apparent loading.

8.2 Enhanced transdermal drug delivery from ethosomes

It is claimed that ethosomes significantly enhance drug delivery across the skin by two principal mechanisms: (a) a fluidizing effect of ethanol on phospholipid bilayers creating a “soft”, deformable vesicle [138, 142-144], and (b) SC lipid disruption by ethanol thereby permitting entry of ethosomes and their associated “payload” into the deeper skin layers (Figure 13) [138].

Delivery of the three aforementioned fluorescent probes into nude mouse skin from ethosomes, liposomes and a hydroalcoholic solution was assessed by LSCM. For both rhodamine red and D-289, optical sectioning of the skin after an 8-hour application clearly

demonstrated that ethosomes were the superior delivery system (Figures 14 and 15). Uptake from the hydroalcoholic solution was greater than that from the liposomes employed. Quantitative analysis of the fluorescence intensity as a function of the depth again revealed the superiority of the ethosomal formulation, in this instance, for calcein and rhodamine red (Figure 16).

Anti-infective drugs when associated with ethosomes appear to be much more efficient delivery systems than classic liposomes. For instance, fluorescently-labeled bacitracin, a polypeptide antibiotic, was delivered from ethosomes to a depth of 200 μm in dermatomed human cadaver skin, whereas its delivery from classic liposomes was negligible [144]. Another study with ethosomal erythromycin demonstrated an improved antibacterial action in comparison to a hydroethanolic solution of the drug. Moreover, an infected skin site treated with the topically applied ethosomal formulation healed as well as that achieved when the drug was systemically injected [145].

Other examples of the efficacy of ethosomal vehicles can be mentioned. Pretreatment of the skin with a formulation containing ammonium glycyrrhizinate (AG), a natural anti-inflammatory agent effective in acute and chronic dermatitis, significantly reduced the intensity and duration of methyl nicotinate-induced erythema in healthy human volunteers, compared to hydroalcoholic solutions of the drug [146].

Ethosomes have also been reported to enhance the percutaneous delivery of ionized drugs, very lipophilic compounds and large hydrophilic molecules. For example, the flux of trihexylphenidyl hydrochloride, an anti-Parkinsonian agent, from an ethosomal vehicle was substantially higher than that from liposomes [142]. Similar results have been reported for propranolol hydrochloride and sodium diclofenac [140]. Ethosomal formulations of testosterone have been compared with marketed transdermal patches, and shown to achieve significantly higher AUC and C_{max} values [138, 147]. Finally, when acyclovir (an effective antiviral drug for the treatment of recurrent herpes labialis infection) was formulated in ethosomes, its delivery resulted in a marked improvement in therapeutic efficiency, compared to the normal treatment (Zovirax[®] cream), presumably as a result of the improved penetration of the active from the lipid-ethanol vehicle [148].

8.3 Summary

Ethosomes have recorded some notable improvements in topical and transdermal drug delivery. While the precise mechanisms of action remain less than fully clear, the combination of a relatively high “dose” of exogenous lipid and ethanol appears to perturb SC barrier function. Whether this approach will ultimately make a significant impact in the clinic requires considerable further work.

9 Niosomes

9.1 Composition and preparation

Niosomes are vesicles prepared from non-ionic surfactants, such as polyoxyethylene alkyl ethers, sorbitan esters, polysorbate-cholesterol mixtures, crown ethers, perfluoroalkyl surfactants, alkyl glycerol ethers, and others [149-153]. The surfactants combine one or more hydrophobic components (e.g., alkyls (C₁₂-C₁₈) or perfluoroalkyls (C₁₀) or a steroidal group) with a hydrophilic head group (e.g., ethylene oxide, glycerol, crown ether, polyhydroxyls, and sugars). The hydrophilic and hydrophobic moieties are linked by ether, ester, or amide bonds.

Niosomes are prepared by similar methods to those used for liposomes, such as hydration of a deposited surfactant/lipid film. This process is usually followed by homogenization, sonication, or extrusion to reduce vesicle size, and then separation of the un-entrapped drug [149, 150, 152]. The stability of niosomal formulations is influenced by factors such as storage temperature, preparation technique, and composition [149, 152]. Vesicle aggregation may be prevented by co-formulation with surfactants. Like liposomes, niosomes can form unilamellar or multilamellar structures, and have the further advantages of improved stability, high purity and low cost.

9.2 Interaction of niosomes with the skin

The interactions of niosomes with the SC have been studied using microscopic techniques. The presence of vesicular structures on or near the surface are clearly seen, but this concentration is quickly attenuated as one examines (e.g., with sequential tape-stripping) the deeper SC layer [29]. The implication is that the niosomes have fused and mixed with the endogenous SC lipids at this point [154, 155]. Although some images of vesicular structures at even deeper regions of the SC have been published, it is impossible to say whether these really represent intact niosomes which have transported down from the skin surface, or whether there has been spontaneous regeneration of a vesicle as the degree of hydration of the SC increases.

9.3 Applications of niosomes in pharmaceuticals and cosmetics

Drugs encapsulated in niosomes, when these vesicles fuse with the SC, may be subjected to enhanced skin penetration due to an altered thermodynamic activity gradient [154, 155], or to the action of the “released” nonionic surfactants on SC barrier function [155, 156]. Enoxacin, for example, was much better delivered from niosomes than either liposomes or when applied as a simple drug solution [154]. If niosomes are used on skin for which the barrier function is significantly less than that typical of intact skin, then even rather large compounds can be delivered. Thus, β -galactosidase and luciferase reporter genes have been successfully transported across rat pup skin from niosome formulations [157]. In addition, and similar to other vesicular carriers, niosomes have been shown capable of drug delivery to hair follicles, with successful results reported for the polypeptides, interferon- α and cyclosporine [158], as well as for minoxidil [14]. From a transdermal standpoint, estradiol has been examined and the nature of the surfactant used to prepare the niosomes clearly has an important effect [159, 160]. Finally, niosomes have been incorporated into cosmetics for several years, and used in a manner not particularly different from liposomes [161].

9.4 Summary

The behaviour and applications of niosomes parallel, in large part, those described for liposomes, although the level of activity, overall, is rather less.

10 Conclusions

Research into nano-sized delivery systems for topical “actives” has been the focus of considerable effort in the pharmaceutical and cosmetic industries for more than twenty years. The composition of different nanoparticles and their methods of preparation show some diversity. However, a size-reduction step with high pressure homogenization, sonication or extrusion is usually required to break some sort of microemulsion into nanoparticles. Depending on their intended use, the resulting carriers can be designed to facilitate or to retard the penetration of drugs and active ingredients into the epidermis or deep dermis and beyond. Rigid nanoparticles, such as liposomes, polymeric nanocapsules and SLN, improve localization of the “active” in the upper skin layers, and may have an ability to form a skin surface film after topical application, thereby preventing water evaporation and increasing skin hydration. Transfersomes and ethosomes are variations of liposomes which incorporate “edge activators” or ethanol to confer the property of ultradeformability. It is claimed that this allows significantly enhanced transport of associated active species into the deeper skin layers via a variety of (not unambiguously proven) mechanisms, including an action as true “carriers”, SC lipid disruption, osmotic gradients, and so on.

There is also a general perception that just about all nano-carriers can “target” appendageal structures, like hair follicles. However, it seems likely that this is a non-specific, physical phenomenon and it remains to be seen whether these observations can be translated into a real, observable benefit.

In conclusion, despite a large and growing scientific and patent literature on the development and evaluation of nanoparticles for the delivery of topical agents, only a few commercial products containing such structures have appeared on the market. While the cosmetic industry has been quick to seize on these novel nanostructures, and to exploit their marketing and sensorial attributes, the pharmaceutical sector has been more reticent and has (currently) demanded quantifiable evidence that these inevitably more expensive products in terms of both materials and manufacture out-perform significantly more conventional formulations. For the moment, at least, this challenge has rarely been met.

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Figure Legends

- Figure 1 Schematic representation of different kinds of nanoparticles.
- Figure 2 Skin permeation pathways.
- Figure 3 Image of a nanoemulsion (15% oil) stabilized with 0.2% carbomer at pH = 7.
- Figure 4 The susceptibility of *Candida albicans* to a X8W₆₀PC nanoemulsion and to its individual ingredients. Cells were treated with either 0.1% X8W₆₀PC nanoemulsion or with individual ingredients at the equivalent concentrations for 15 minutes at 37°C. After treatment, cells were washed and plated on BH1 plates to assess the number of CFU. The bars represent standard error. D1, D2, and D3 depict three detergents used to prepare X8W₆₀PC.
- Figure 5 Schematic illustration of liposomes of different size and number of lamellae.
- Figure 6 Effect of different formulations on the flux of caffeine through hairless mouse skin (3% caffeine in each system).
- Figure 7 *Left panel:* laser scanning confocal microscopy image of nude mouse skin *in vivo* (~200 μm^2 area). *Right panel:* Distribution of a fluorescent penetrant in the so-called intercluster (black) and intercorneocyte (grey) regions of the skin.
- Figure 8 Chemical structure of PVA-FA derivatives.
- Figure 9 Amount of benzophenone-3 absorbed across porcine ear skin *in vitro* (Mt), relative to the applied quantity (Mo), as a function of time following administration of various PVA-fatty acid nanocapsule-containing suspensions. The highest degree of transport occurred when the sunscreen was applied in solution in the absence of nanocapsules.
- Figure 10 Relative occlusivity of tocopherol acetate-based SLN and a reference emulsion after 6, 24 and 40 h post-application.
- Figure 11 Scanning electron micrograph of a dried SLN film on a single tape.
- Figure 12 Entrapment of three fluorescent probes by liposomes (a-c) and ethosomes (d-f).

The fluorescent probes were rhodamine red (a, d), D-289 (b, e) and calcein (e, f). White represents the highest concentration of probe, with yellow and red signifying progressively lower levels.

Figure 13 Proposed mechanisms for enhanced drug delivery across the skin from ethosomal vehicles.

Figure 14 LSCM images of rhodamine red penetration into nude mouse skin following an 8 hour application of (a) ethosomes, (b) a hydroalcoholic solution of the dye, and (c) liposomes.

Figure 15 LSCM images of D-289 penetration into nude mouse skin following an 8-hour application of (a) liposomes, (b) a hydroalcoholic solution of the dye, and (c) ethosomes.

Figure 16 Skin penetration profiles of (A) calcein, and (B) rhodamine red, following their application to hairless mouse skin from either ethosomes, liposomes or a hydroethanolic solution for 8 hours. Depth of skin penetration and fluorescence intensity were determined by LSCM. Significantly different values (* $p < 0.001$, ** $p < 0.05$; ethosomes versus other systems) are highlighted.

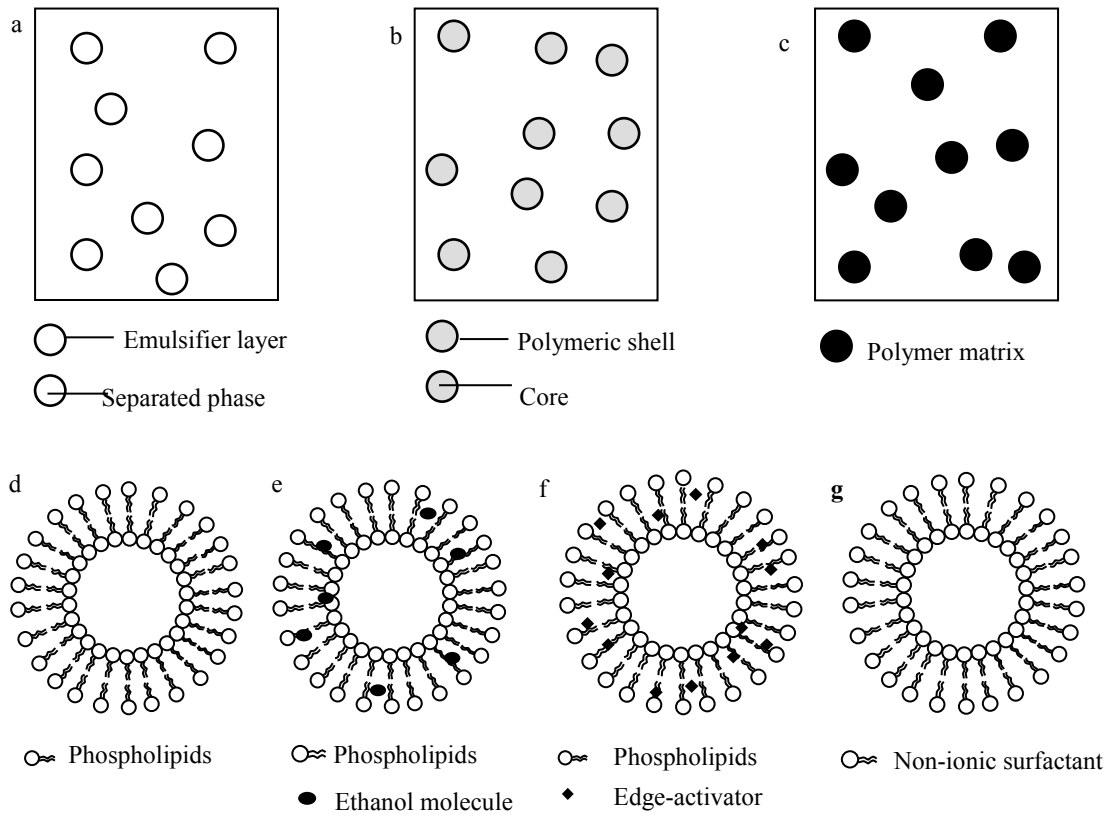


Figure 1: Schematic representation of (a) a nanoemulsion, (b) polymeric nanocapsules, (c) polymeric nanospheres, (d) individual liposomes, (e) an ethosome, (f) a transfersome, and (g) a niosome.

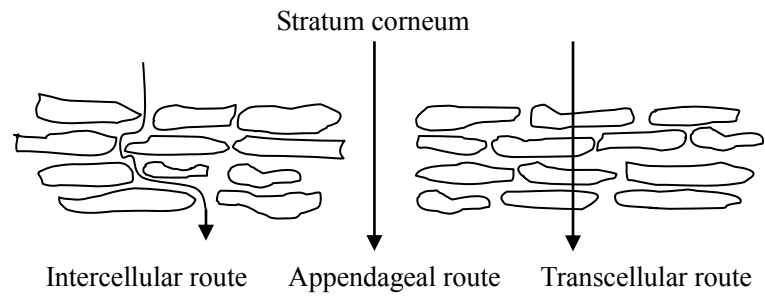


Figure 2: Skin permeation pathways.

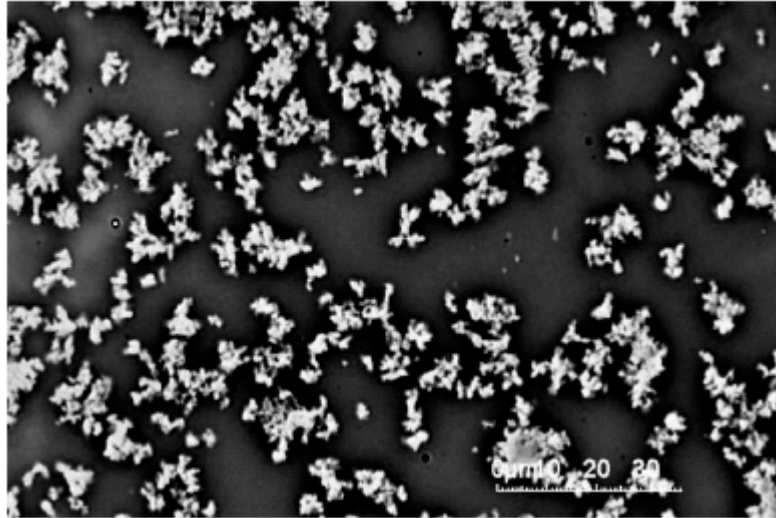


Figure 3: Image of a nanoemulsion (15% oil) stabilized with 0.2% carbomer at pH = 7.
Adapted from reference [35].

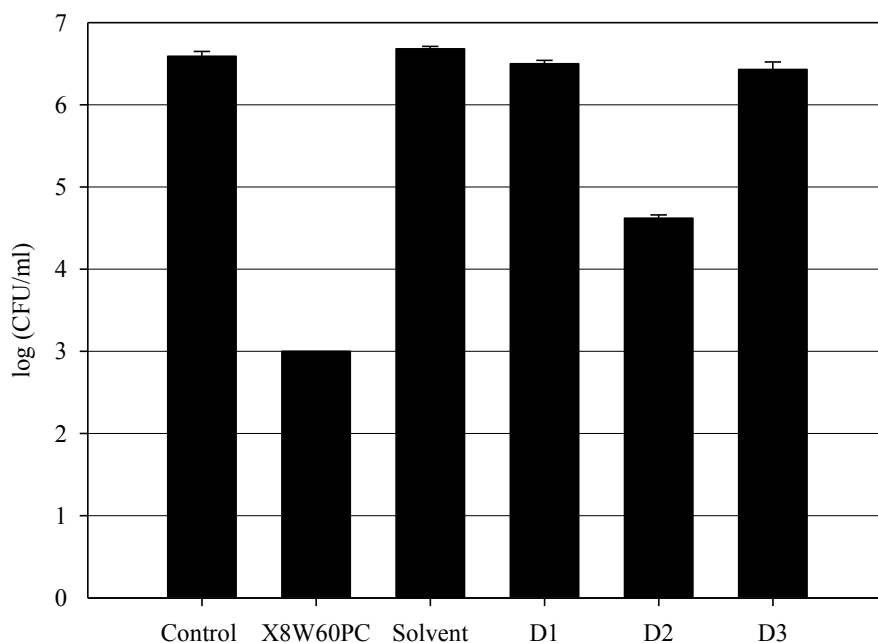


Figure 4: The susceptibility of *Candida albicans* to a X8W₆₀PC nanoemulsion and to its individual ingredients. Cells were treated with either 0.1% X8W₆₀PC nanoemulsion or with individual ingredients at the equivalent concentrations for 15 minutes at 37°C. After treatment, cells were washed and plated on BH1 plates to assess the number of CFU. The bars represent standard error. D1, D2, and D3 depict three detergents used to prepare X8W₆₀PC. Redrawn from reference [41].

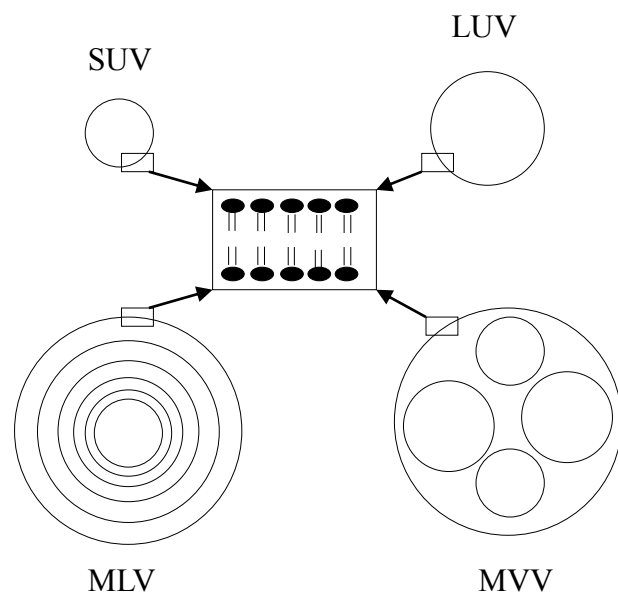


Figure 5: Schematic illustration of liposomes of different size and number of lamellae.

Redrawn from reference [33].

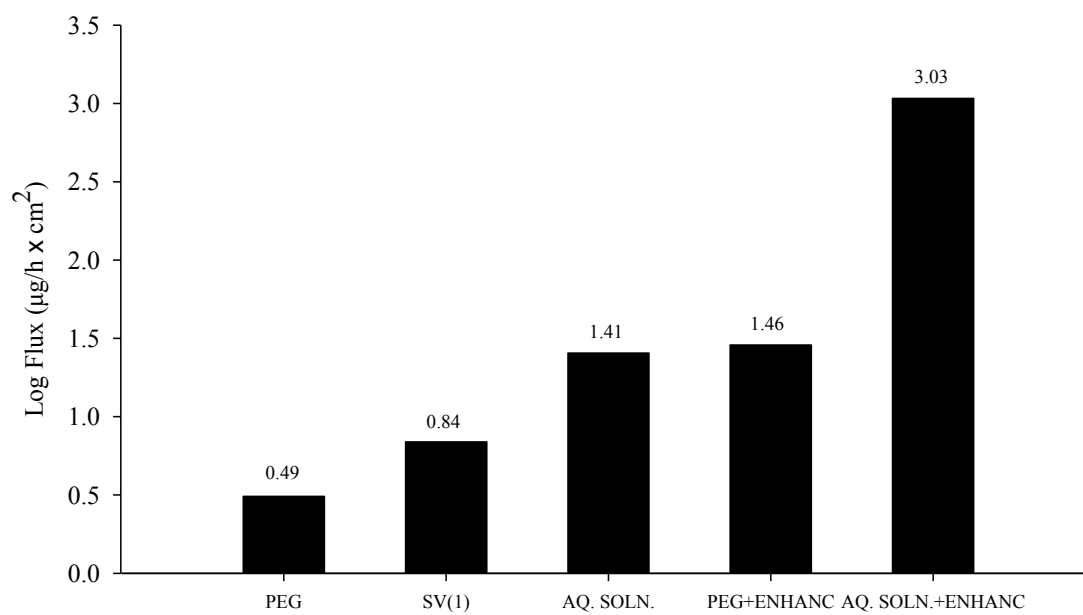


Figure 6: Effect of different formulations on the flux of caffeine through hairless mouse skin (3% caffeine in each system). Adapted from reference [65].

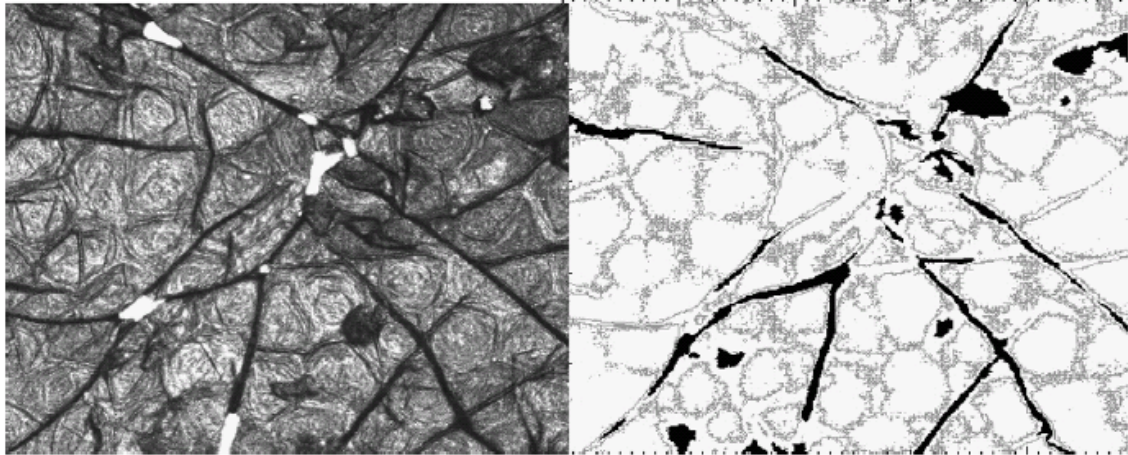


Figure 7: Left panel: laser scanning confocal microscopy image of nude mouse skin *in vivo* ($\sim 200 \mu\text{m}^2$ area). Right panel: Distribution of a fluorescent penetrant in the so-called intercluster (black) and intercorneocyte (grey) regions of the skin. Reprinted from reference [79].

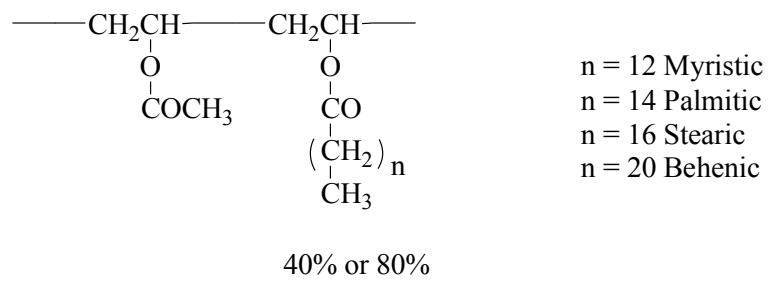


Figure 8: Chemical structure of PVA-FA derivatives. Redrawn from reference [97].

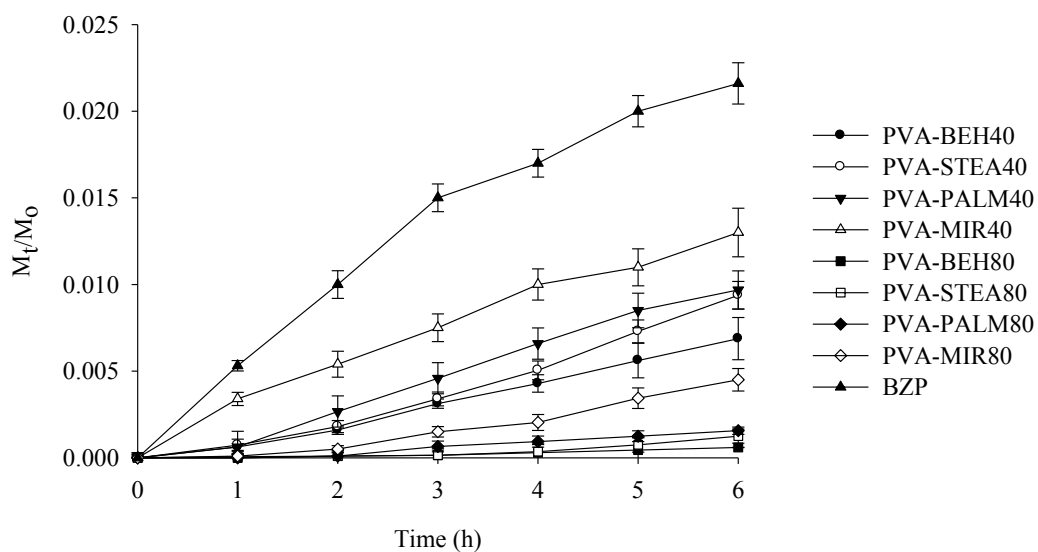


Figure 9: Amount of benzophenone-3 absorbed across porcine ear skin *in vitro* (M_t), relative to the applied quantity (M_0), as a function of time following administration of various PVA-fatty acid nanocapsule-containing suspensions. The highest degree of transport occurred when the sunscreen was applied in solution in the absence of nanocapsules. Redrawn from reference [97].

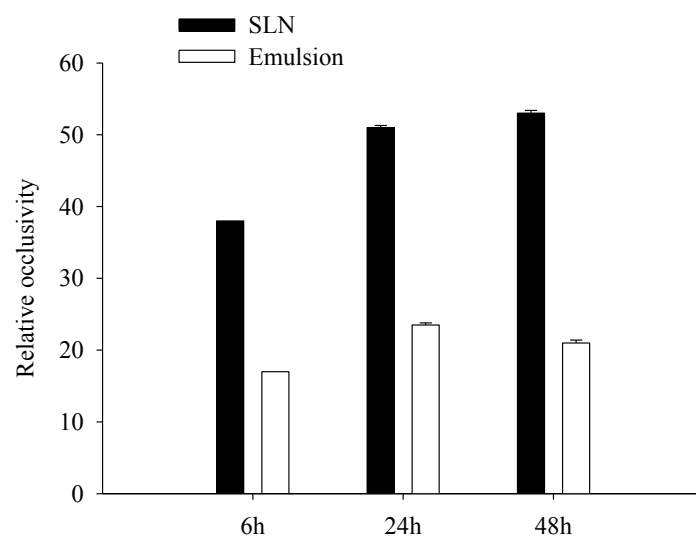


Figure 10: Relative occlusivity of tocopherol acetate-based SLN and a reference emulsion after 6, 24 and 40 h post-application. Redrawn from reference [123].

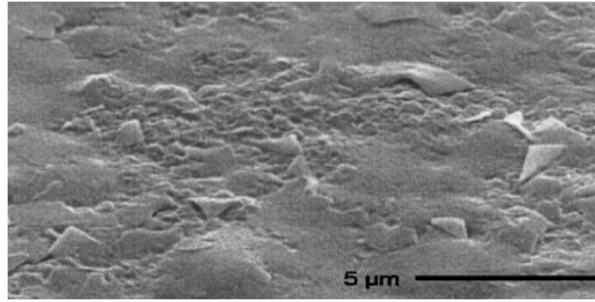


Figure 11: Scanning electron micrograph of a dried SLN film on a single tape. Reprinted from reference [123].



Figure 12: Entrapment of three fluorescent probes by liposomes (a-c) and ethosomes (d-f). The fluorescent probes were rhodamine red (a, d), D-289 (b, e) and calcein (e, f). White represents the highest concentration of probe, with yellow and red signifying progressively lower levels. Reprinted from reference [138].

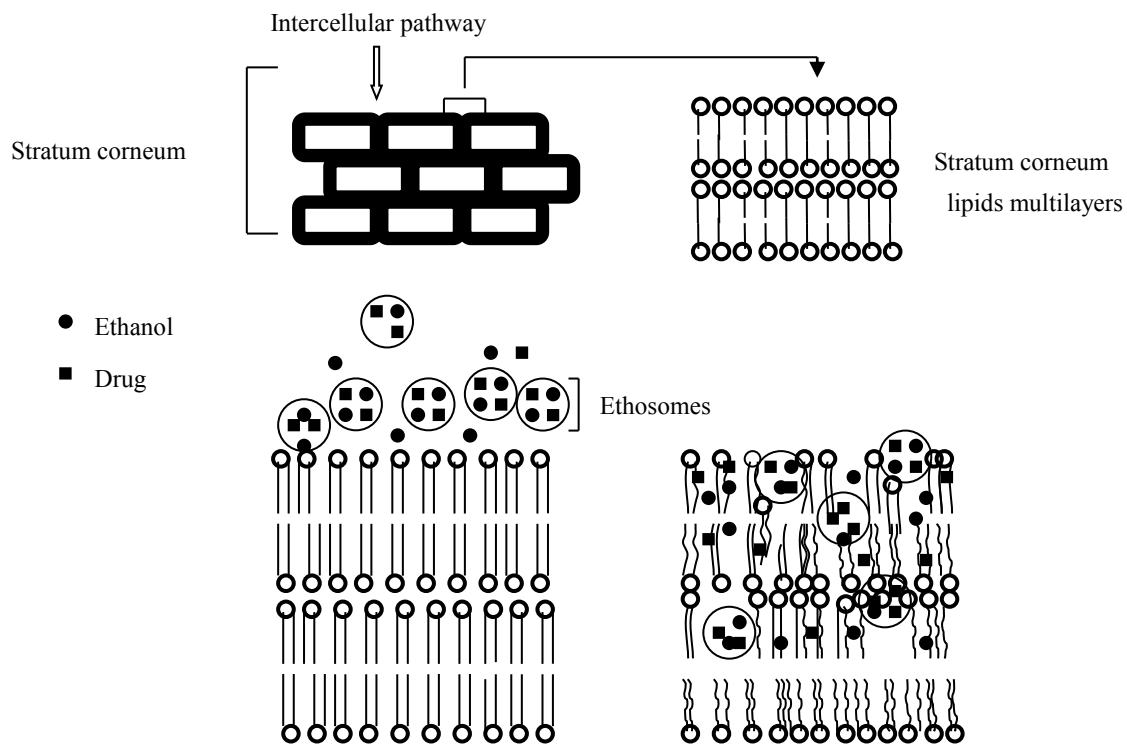


Figure 13: Proposed mechanisms for enhanced drug delivery across the skin from ethosomal vehicles. Adapted from reference [138].

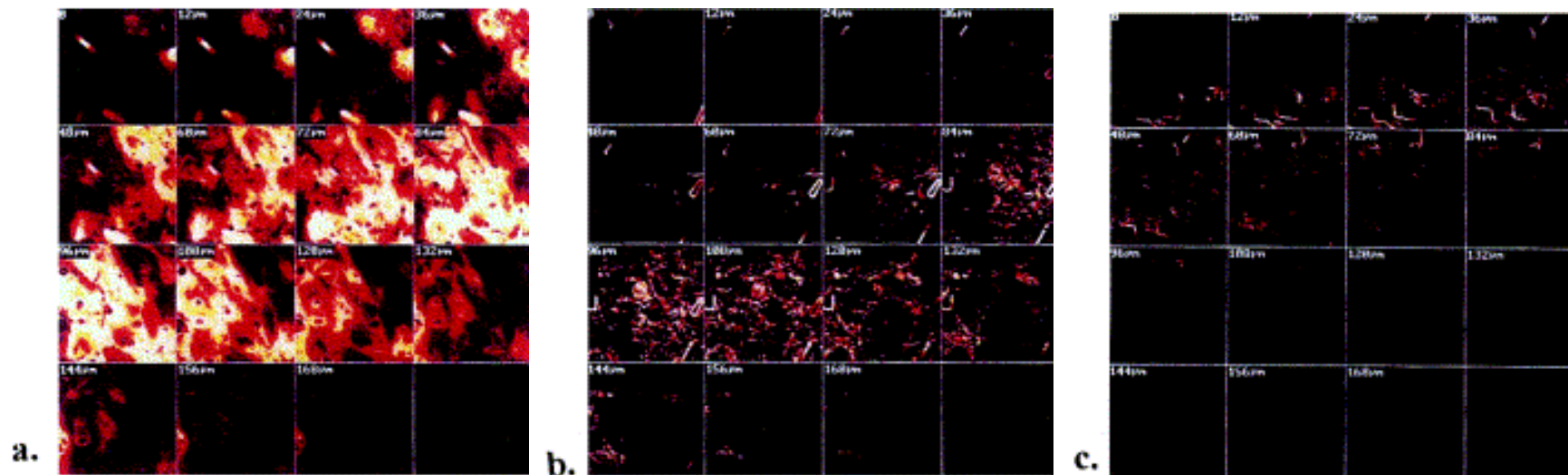


Figure 14: LSCM images of rhodamine red penetration into nude mouse skin following an 8 hour application of (a) ethosomes, (b) a hydroalcoholic solution of the dye, and (c) liposomes. Reprinted from reference [138].

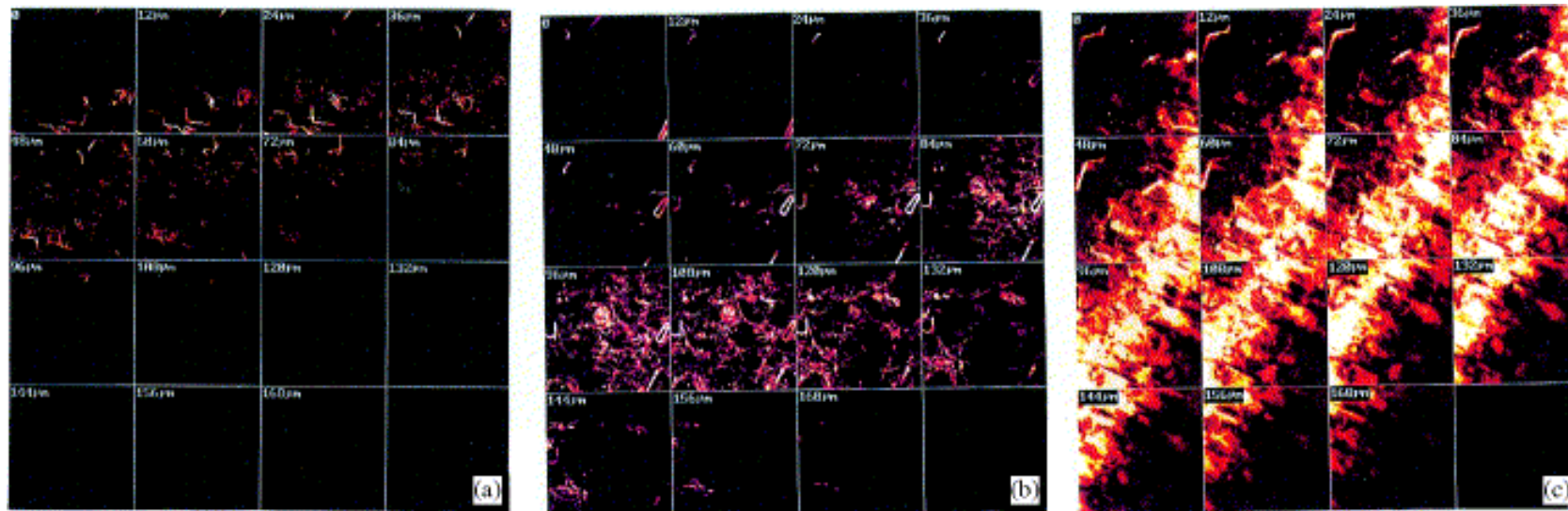
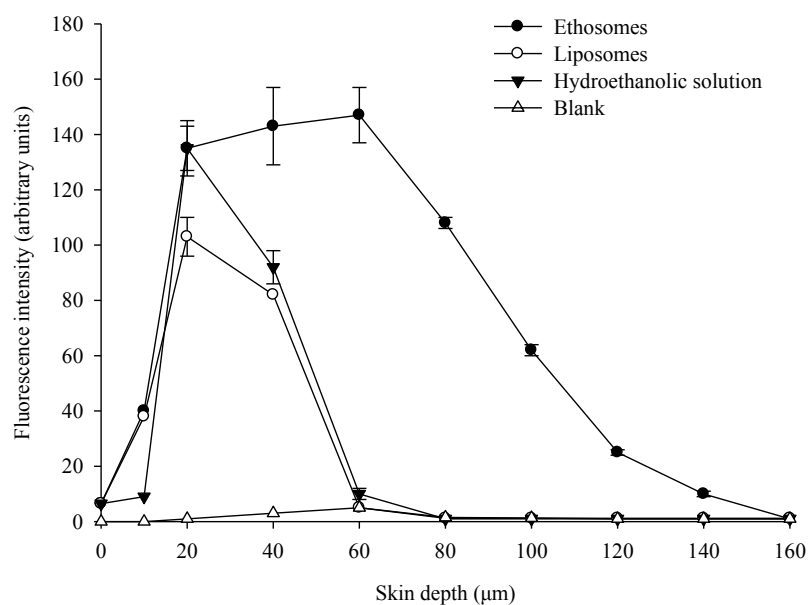
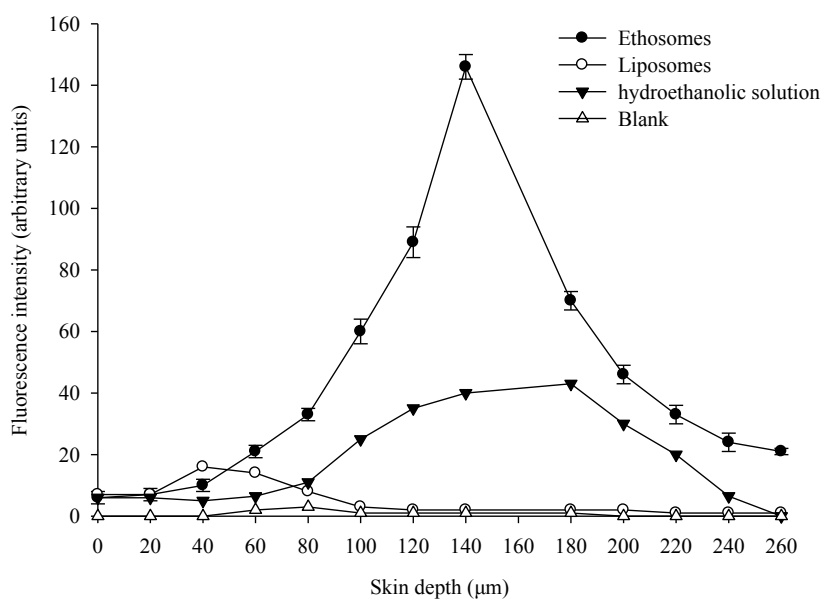


Figure 15: LSCM images of D-289 penetration into nude mouse skin following an 8-hour application of (a) liposomes, (b) a hydroalcoholic solution of the dye, and (c) ethosomes. Reprinted from reference [142].



(A)



(B)

Figure 16: Skin penetration profiles of (A) calcein, and (B) rhodamine red, following their application to hairless mouse skin from either ethosomes, liposomes or a hydroethanolic solution for 8 hours. Depth of skin penetration and fluorescence intensity were determined by LSCM. Significantly different values (* $p < 0.001$, ** $p < 0.05$; ethosomes versus other systems) are highlighted. Redrawn from reference [162].