Expert Opinion

Colloidal carriers for the enhanced delivery through the skin

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Background: The skin is the largest organ of our body and acts as a protective barrier with sensory and immunological functions. Its peculiar structure influences the passage of bioactives and only its modulation can facilitate the drug dermal/transdermal diffusion. In the past few years research in this field has assured better use of this application area. *Methods*: One of the most promising approaches is the use of drug delivery devices; this review explains the state of the art of drug transport through the skin by means of vesicular (classic liposomes, Transfersomes, niosomes and ethosomes) and particulate systems. *Results/conclusion*: Colloidal drug delivery systems are important in the field of drug delivery systems as their different characteristics make them suitable for various purposes.

Keywords: ethosomes, liposomes, niosomes, polymeric particles, skin delivery, solid lipid nanoparticles, transfersomes

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1. Introduction

The skin is the largest organ of our body and represents a remarkable barrier which protects us from external agents [1]. It is made up of three main layers, namely the epidermis (divided into the stratum corneum and the viable epidermis), the dermis and the hypodermis. In particular, the stratum corneum is characterized as having a thickness of 10 – 20 µm and contains between 10 and 15 layers of corneocytes which are continually being removed and regenerated, while the viable epidermis consists of multiple layers of keratinocytes at various stages of differentiation. The lipophilic nature of the stratum corneum (rich in phospholipids, ceramides, cholesterol and cholesterol esters) influences the penetration of active compounds, and percutaneous drug absorption is evaluated by means of Fick's Law [2]:

$$J = \frac{dQ}{dt} = \frac{K_s \times D}{h} \times C \times A$$

where dQ/dt is the amount of drug diffused per unit of time or drug flux (J), K_s is the partition coefficient, D is the diffusion coefficient, h is the thickness of the stratum corneum, ΔC is the concentration of the active compound and A is the skin surface area utilized for drug administration.

In recent years, the investigation of percutaneous routes of drug permeation has been improved in two fundamental ways: firstly, this mode of drug administration is able to assure physicochemical protection for many drugs such as peptides and proteins (which is better than that of alternative methods of administration, i.e., oral), and secondly, it improves patient compliance. Moreover, the choice of a formulation can favor the targeting of the active compounds in the skin strata or in the circulatory system. It is very difficult to deliver a drug compound through the skin, especially if it has hydrophilic physicochemical properties. Therefore,

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substances are normally used that improve the percutaneous diffusion, called penetration enhancers (i.e., ethanol). Unfortunately, these substances increase the percutaneous permeation of all the components, causing an increase in the collateral effects related to some substances (surfactants, preservatives etc.). To avoid these problems, innovative drug delivery systems have been developed. In this review, we explain the state of the art of the foremost carriers used to increase the amount of percutaneous permeation of active drug compounds. In particular, we describe the evolution of vesicular carriers (from liposomes/niosomes to Transfersomes® [IDEA AG], ethosomes and solid lipid nanoparticles), plus the use of polymeric micro and nanoparticles for topical application.

2. Colloidal carriers used in topical applications

2.1 Liposomes

The appearance of Banghman's vesicles in 1960 (the so-called liposomes) represents an innovative approach in the field of drug delivery systems. Liposomes are mostly made up of phospholipids, and for this reason they are highly biocompatible and biodegradable, but they can also contain other components (i.e., steroid molecules, gangliosides, polymeric materials) that modify their biodistribution, drug release profile and clearance. These carriers have the ability to delivery macromolecules with different physicochemical characteristics, thus permitting a selective therapeutic effect [3]. Biocompatibility and biodegradability of the different components of liposomes give colloidal devices a safety profile which enables them to be used as a drug delivery system in the pharmaceutical field [4]. Many scientists are focused on the possibility of liposomes being used to administer drugs by several routes, such as oral, parenteral and topical [5-7]. In this investigation we have found that the similarity of the bilayer structure of lipid vesicles and that of the natural membrane (besides the ability of colloidal drug carriers to modify cell membrane fluidity and to fuse with cells) suggests that liposomal formulations exhibit interesting properties for enhancing the dermal and transdermal delivery of drugs and can represent an alternative route to oral and parenteral drug administration [8-10]. The potential application of liposomes in skin delivery, compared with conventional non-vesicle formulations, has been proposed because of the extremely promising properties of vesicular systems in terms of drug penetration enhancement [11,12], improved pharmacological effects [13,14], decreased side effects, controlled drug release [15] and drug photoprotection [16]. The penetration behavior of drug-loaded liposomes through the skin is based on the technological parameters of colloidal devices. In particular, preparation methods, vesicle-membrane structure (uni- or multi-lamellar), lipid composition, mean sizes and physicochemical properties of drugs must be investigated when liposomal formulations are used as topical devices [11,12].

Different interpretations have been proposed concerning the mechanism of drug permeation through the skin in the case of liposomal formulations [17]. The theories are as follows: liposomes penetrate intact into the skin [18,19]; vesicles are disintegrated on the skin surface and penetrate as individual lipid molecules through the stratum corneum, thus producing fluidization and modification of the wall-thickness barrier [20]; liposomes are absorbed on the skin surface and then fused with lipids in the stratum corneum, thus promoting a lipid exchange between phospholipids of bilayer and cellular skin lipids [21,22], allowing a direct transfer of the drug to the stratum corneum; and liposomes have an occlusive effect on the stratum corneum [23].

Differences in permeation behavior of liposomes through the skin can be explained by interaction models between vesicles and lipids of the stratum corneum. This effect is mediated by the physicochemical properties of colloidal devices. In particular, vesicle–lipid compositions, besides being able to modulate the elasticity and the thermodynamic state of the liposomal bilayer, are also able to modulate the trans-dermal permeation of drug compounds encapsulated in liposome devices [24,25]. Recently Sinico *et al.* [26] showed that the diffusion of tretinoin through artificial and natural membranes is influenced by liposome composition and structure. In this case, unilamellar vesicles permitted greater drug release than multilamellar vesicles. Moreover, the presence of Phospholipon 90® (PL90, Rhône-Poulenc RorerPL90) in a liposome composition, when compared with hydrogenated Phospholipon 90H (PL90H), increased the amount of tretinoin which permeated through the silicon membrane. Experimental findings showed that (except in the presence of multi-lamellar vesicles containing PL90 in the lipid composition) the presence of a negative charge (diacetylphosphate) in the colloidal vesicles improved skin permeation of the drug compared with the presence of a positively charged inducer (stearylamine). This effect could be explained as a consequence of the formation of pairs of ions between the drug compound and the positively charged lipid.

In vitro permeation experiments using newborn pig skin and Franz diffusion cells showed that permeation profiles during 9 h of incubation do not show a classic steady-state profile for all tested formulations. The flow of tretinoin that permeates through the skin during the first 3 h of incubation is rapid and then slows down between the third and the ninth hours. The results highlighted the fact that the incidence of permeation of the drug compound encapsulated in liposomes is greater when liposome vesicles containing stearylamine are present in the lipid composition; however, a reduction of the amount of drug compound permeated through the skin and consequently a plateau-like permeation profile can be obtained for negatively charged PL90 liposomes.

The fluidity of the bilayer of many liposome formulations simultaneously containing cholesterol, tretinoin and soy-hydrogenated phosphatidylcholine is modified when the experimentation temperature is increased. The lipid

composition of liposomal formulations increased the amount of molecules which permeated through the skin when compared with a commercial formulation (Retin-A®, Ortho Dermatological Division, Ortho-McNeil Pharmaceutical, Inc.), a hydroalcoholic solution and an oil solution, suggesting that the highest accumulation values are obtained for dicetylphosphate liposomes and these values are independent of mean sizes and lamellar structure of vesicles. **Figure 1** highlights the fact that liposomal formulations do not penetrate intact into the deeper skin layers and suggests that absorption, lipid exchange and fusion are probably involved in the permeation of vesicles across the skin. This, in turn, suggests that the main advantage of liposomes as a transdermal drug delivery system is due to the drug residence time on the skin surface.

Another important parameter to be observed for trans-dermal liposome delivery is the thermodynamic state of the liposomal bilayer. It is generally reported that the incorporation of a drug compound into liposomes in a gel state permits a slower skin permeation rate than that for vesicles in a fluid state. The thermodynamic behavior of colloidal vesicles is influenced by phospholipid and cholesterol percentages. Different reports have shown that the transition temperature of lipid components may be able to modulate dynamic behavior of colloidal vesicles from the gel state to the liquid state [24]. At the same time, a percentage of cholesterol (in the range of 30 – 50% of the mass of the liposome membrane) represents an optimal value in the case of liposomes for topical application.

In another study, Betz *et al.* [27] investigated the influence of mean size and polidispersity distribution of colloidal devices in the case of a liposomal suspension for topical application. Generally, average dimensions and polidispersity indices of vesicle formulations are influenced by lipid compositions, suggesting that the enhancing effect on the penetration of liposomal devices appears to be a function of phospholipidic origin. Data obtained from Betz and his co-workers show that liposomes prepared using soy phospholipids increased mean sizes as a consequence of the increase in the phospholipid concentration, whereas colloidal vesicles containing egg phospholipids in the bilayer are smaller in size and present a more homogeneous distribution. Modification of the physicochemical parameters of colloidal vesicles depends on the percentage of phospholipids of different origins used in the formulation and is influenced by the composition of the aqueous solution used in the formulation. In this case, it is noteworthy that liposomes prepared with egg phospholipids (2%) and soy phospholipids (2%) have shown particle sizes in the same range (228.2 – 261.9 nm), whereas vesicle suspensions prepared from soy phospholipids (1%) are up to 100 nm smaller, irrespective of the dilution media. Moreover, the composition and preparation process of the formulations have been optimized with respect to the homogeneity and stability of the liposomes. The freeze fracture transmission electron microscopy analysis reported in **Figure 2** highlights the fact that liposomes made using egg phospholipids have a larger diameter (1 – 3 μm) and a nonhomogeneous distribution compared with those obtained using soy phospholipids [27]. The differing behaviors of the liposomes are probably correlated to the phase transition temperature of lipid materials used to prepare the colloidal devices. The presence of free fatty acids in the liposomal formulation, which is able to reduce the phase transition temperature of liposomes, is an advantage for vesicular suspensions because this affects the penetration depth of the system when it is used for transdermal application.

The penetration behavior of liposomal suspensions is also influenced by the water content of the skin. The effect of water content in the skin surface can be determined by measuring transepidermal water loss. Measurements of skin water content in male and female volunteers were carried out by Betz *et al.* [27]. Experimental data have shown that skin water content remains significantly higher for formulations prepared using egg phospholipids (1 and 2%) and soy phospholipids (1%) when compared with the control solution (**Figure 3** and **Table 1**). A greater increase in skin water content is obtained using PLE formulations, 1 and 2%, respectively, suggesting that the multilamellar structure of egg liposomes has a positive influence on the increase in skin water content, whereas soy phospholipid formulations do not show any advantages in terms of skin water content according to recent references.

The addition of a gelling agent (Carbopol®, Noveon) to the liposomal formulation modified the viscosity of the colloidal vesicles compared with the control gel formulation. The presence of Carbopol produced a contradictory effect on the rheological properties of the colloidal suspensions. In fact, the liposomal formulations exhibited a decrease in viscosity compared with the control gel when 0.1% w/w of gelling agent was added to the formulation, and an increase in viscosity for 0.3 and 0.5% w/w of Carbopol. The results concerning the viscosity of the liposomal formulation are in agreement with those obtained for gel strength. Viscosity and gel strength influence the drug deposition of gel-liposomal formulations into the skin [28]. Specific investigations have shown that maximum drug deposition is obtained for a liposomal gel containing 0.3% w/w of Carbopol, suggesting that a copolymer block of Carbopol can diminish the release of a drug compound from the liposomal suspension and increase its viscosity.

Recently [29] a multi-carrier system was developed. Liposomes, made up of phosphatidylcholine and cholesterol, were prepared using different methods, namely thin layer evaporation, freezing and thawing, extrusion and reverse-phase evaporation techniques, thus obtaining different kinds of vesicles; that is, multilamellar (MLV), frozen and thawed multilamellar (FATMLV), small unilamellar (SUV) and large unilamellar (LUV) vesicles, respectively. Ketoprofen-hydroxypropyl–β-cyclodextrin complex was selected as the drug solution used to increase encapsulation efficacy of the active compound. The encapsulation efficacy of the drug

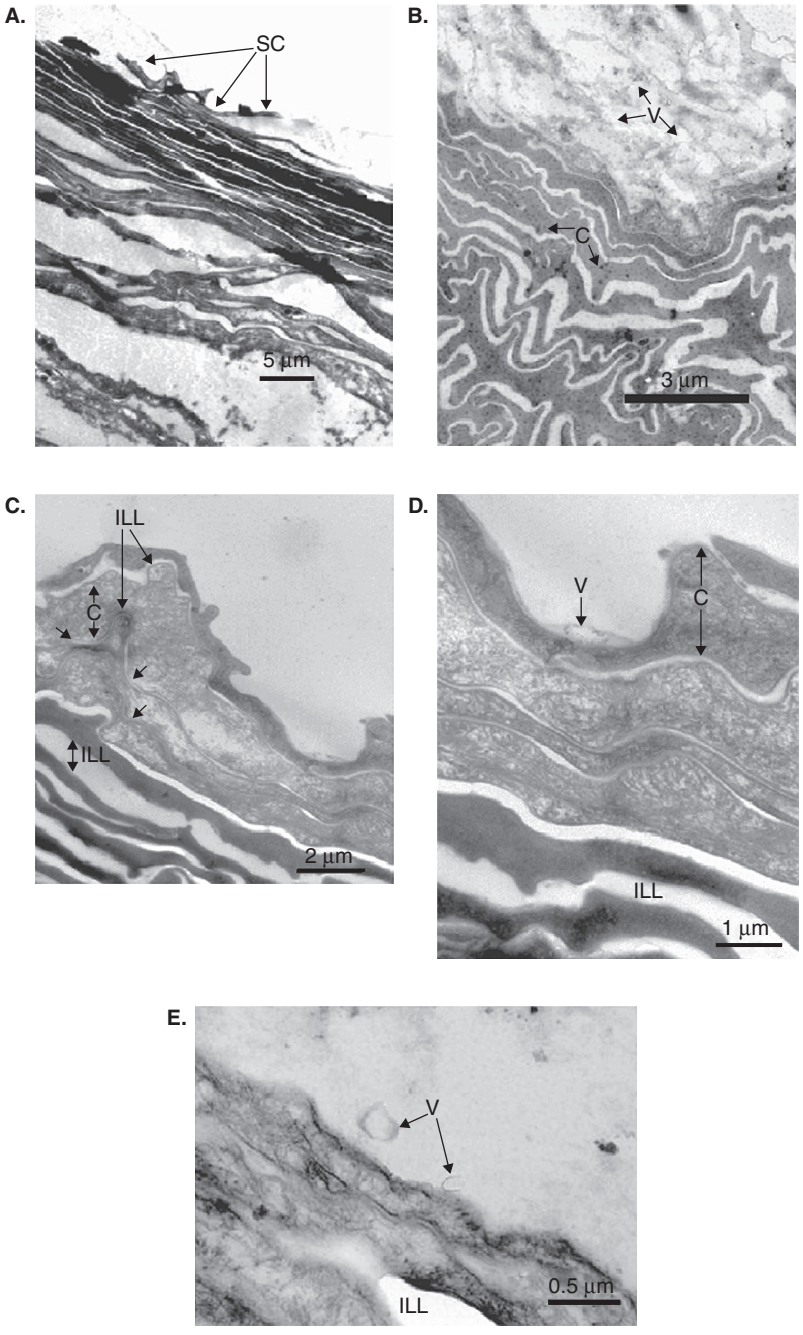

Figure 1. Transmission electron micrographs of pig skin treated with negatively charged liposomes or PBS solution (control) in occlusive conditions. A. Overview of control pig skin incubated for 1 h with PBS. **B.** Pig skin after 1 h of treatment with P90H/DCP liposomes. Some aggregates of vesicles (V) can be seen intact on the stratum corneum (SC) surface; corneocytes (C) are swollen and less electron dense than the control, while intercellular lipids (ILLs) are larger, with an irregular appearance. **C.** Overview of the pig skin treated with P90/DCP liposomes (1 h). Outermost corneocytes are very swollen and not as dark as the control. Cellular cornified envelope (arrows) can also be observed. Inner stratum corneum layers show intercellular lamellar lipid regions (ILL_1) larger than those of the upper four stratum corneum layers. **D.** Same sample as C at higher magnifications. One liposome is spread on the corneocyte surface and is fusing with it. **E.** Outermost stratum corneum after 9 h of treatment with P90H/DCP liposomes. Two liposomes are still adhering to the pig skin surface.

Reprinted from Sinico C, Manconi M, Peppi M, et al. Liposomes as carriers for dermal delivery of tretinoin: in vitro evaluation of drug permeation and vesicle-skin interaction. J Control Release 2005;103:123-36, Copyright (2005), with permission from Elsevier.

DCP: Dicetylphosphate; P90: Soy phosphatidylcholine; P90H: Hydrogenated soy phosphatidylcholine.

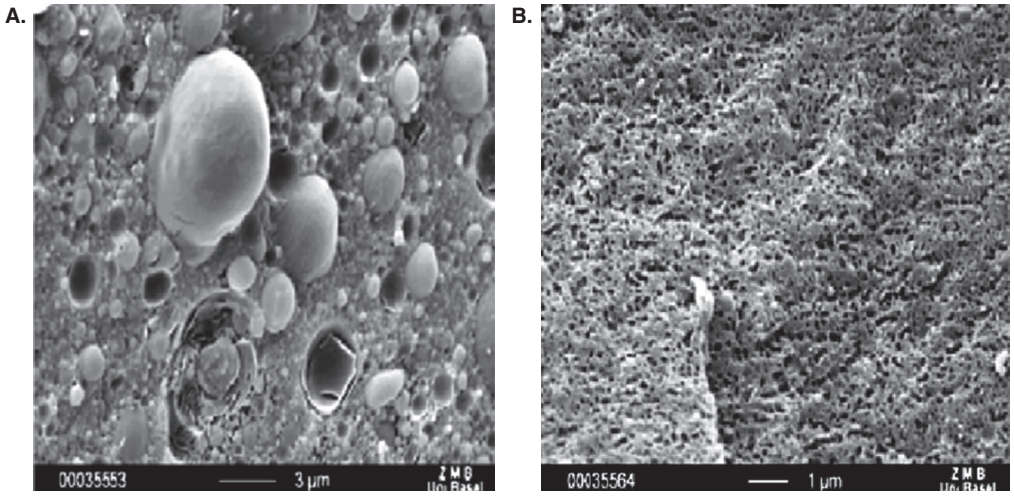

Figure 2. Characterization of liposomes by freeze fracture technique. Magnification 10,000. **A.** Liposomes prepared from egg phospholipids (PLE 1%). **B.** Liposomes prepared from soya phospholipid (PLS 2%).
Reprinted from Betz G, Aeppli A, Menshutina N, Leuenberger H. In vivo comparison of various liposome formulations for cosmetic application. Int J Pharm 2005;296:44-54, Copyright (2005), with permission from Elsevier.

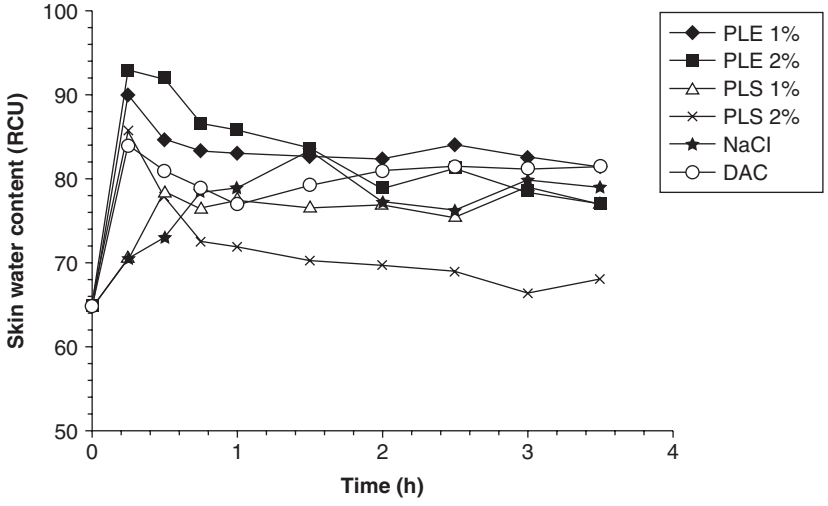

Figure 3. Skin water content measurement after single application of six formulations averaged over all 10 volunteers.
Reprinted from Betz G, Aeppli A, Menshutina N, Leuenberger H. In vivo comparison of various liposome formulations for cosmetic application. Int J Pharm 2005;296:44-54, Copyright (2005), with permission from Elsevier.
RCU: Relative corneometer units.

complex and mean sizes of liposomal formulations were greatly influenced by the preparation procedure, and drug loading was increased by improving the concentration of the complex for MLV and LUV vesicles, while an inverse effect was obtained for FAT–MLV. Another important parameter investigated was the number of lamella in the liposome formulations prepared by using different procedures in the presence of a selected concentration of drug–cyclodextrin complex. Images obtained using confocal laser scanning microscopy (CLSM) showed that MLV vesicles presented a typically concentric lamellar structure (**Figure 4A**) with respect to the FAT–MLV vesicles (**Figure 4B**), which showed a less regular morphology because of the traumatic effect of the freezing and thawing preparation procedure. No morphological or structural differences were observed in the presence of differing concentrations of ketoprofen-hydroxypropyl–β-cyclodextrin complex entrapped in the liposomes, confirming the fact that the presence of β-cyclodextrin does not affect the lamellar structure of the liposome.

The drug permeability profile for the ketoprofen–β-cyclodextrin complex entrapped in liposomes (across an artificial lipophilic membrane and excised rat skin) was influenced by encapsulation efficacy, liposome dimension and morphological properties of the formulation. In particular, the permeation of liposomes across the skin was

Table 1. Statistical evaluation of the results of skin water content compared with untreated skin, t = 0.

Time (h)	PLE 1% (RCU)	PLE 2% (RCU)	PLS 1% (RCU)	PLS 2% (RCU)
0	64.8	65.0‡	64.9	64.9
0.25	90.1‡	93.0‡	70.5	85.7‡
0.50	84.8‡	92.1‡	78.4‡	78.1*
0.75	83.5‡	86.7‡	76.4‡	72.7
1.0	83.2‡	85.9‡	77.5‡	72.0
1.5	82.8‡	83.7‡	76.5‡	70.3
2.0	82.5‡	79.0*	76.9‡	69.8
2.5	84.2‡	81.4‡	75.4‡	69.0
3.0	82.6‡	78.6*	79.3‡	66.3
3.5	81.6‡	77.1*	77.0‡	68.1

Data from Betz *et al.* [27].
*$p < 0.05$.
‡$p < 0.01$.
RCU: Relative corneometer units.

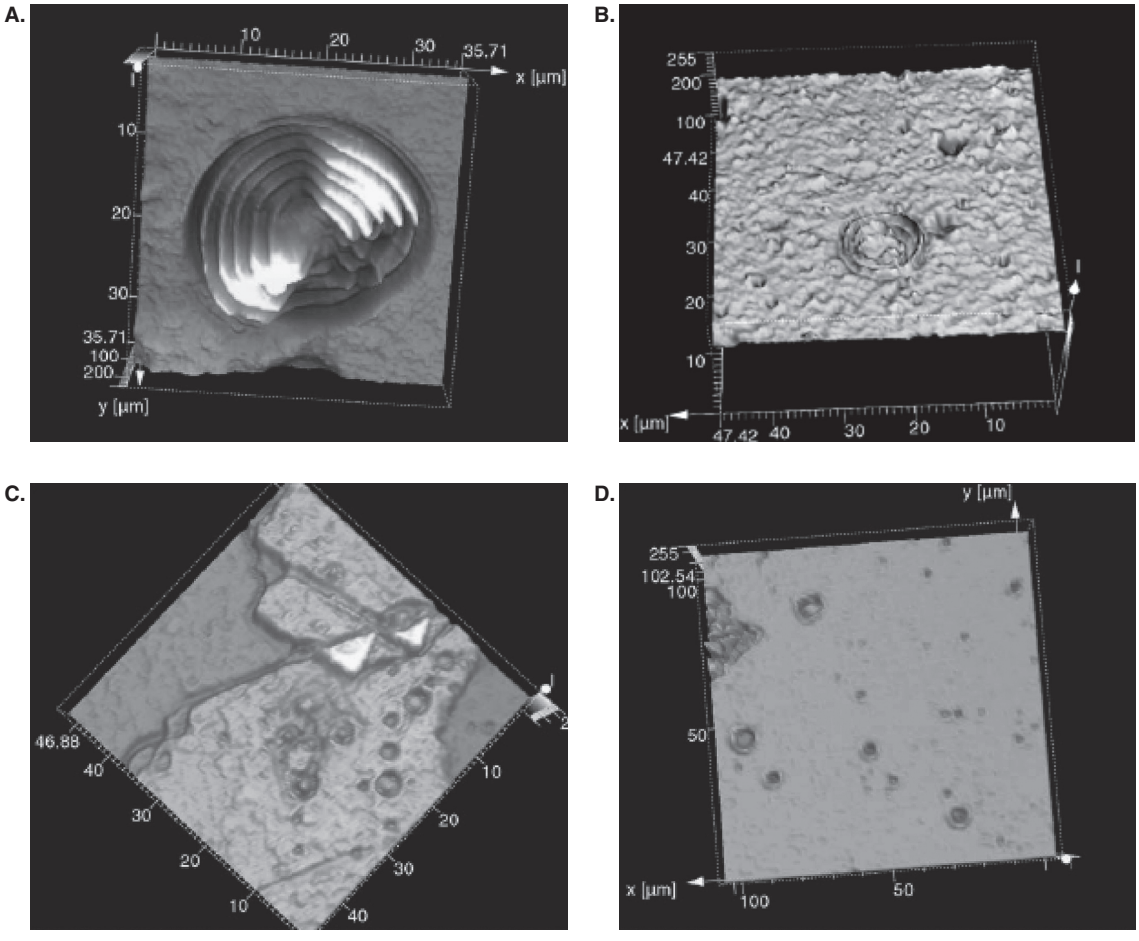

Figure 4. Transmission tridimensional confocal laser scanning microscopy images of different kinds of liposomes all prepared in the presence of 5 mM ketoprofen–HP-β-Cyd complex. A. Multi-lamellar vesicle. **B.** Frozen and thawed multi-lamellar vesicle. **C.** Large unilamellar vesicle. **D.** Small uni-lamellar vesicle.
Reprinted from Maestrelli F, Gonzalez-Rodryguez ML, Rabasco AM, Mura P. Effect of preparation technique on the properties of liposomes encapsulating ketoprofen-cyclodextrin complexes aimed for transdermal delivery. Int J Pharm 2006;312:53-60, Copyright (2006), with permission from Elsevier.

characterized by the following trend: ketoprofen > ketoprofen-hydroxypropyl–β-cyclodextrin > ketoprofen–β-cyclodextrin. Differences in the permeation rate are correlated to preparation methodologies used to obtain the liposomal suspensions.

2.2 Niosomes

Niosomes are nonionic surfactant vesicles, formed from the self-assembly of nonionic amphiphiles in aqueous media. Niosomes present a bilayer structure similar to that of liposomes. Niosomes are able to entrap hydrophilic and hydrophobic molecules and can be used as a drug delivery system. Low cost, stability and great availability of surfactants has led to the investigation of these colloidal carriers as an alternative to conventional liposomes [30]. Niosomes were used in the early 1970s as a drug delivery system for cosmetic application [31]. The multiple effects of reduction of drug toxicity, modification of pharmacokinetics plus bioavailability of drug compounds seems to indicate that nonionic surfactant vesicles are interesting drug delivery systems for use in topical application [32-34].

The topical application of niosomes can increase the residence time of the drug in the stratum corneum, allowing the epidermis to receive a local therapeutic effect, thus reducing the systemic absorption of the drug compound. At the same time, the enhancing effect of niosomes on skin penetration after topical application reduces transepidermal water loss and increases smoothness through the replenishment of lost skin lipids [35]. Moreover, the structures of nonionic surfactant vesicles offer great opportunities for drug delivery because their size, shape, surface charge, lamellae nature and composition can be altered [36]. The characteristics of niosomal suspensions are able to modify vesicle–skin interaction and the therapeutic efficacy of vesicular formulations, particularly the degree of elasticity and the thermodynamic behavior of the lipidic components of the bilayer [30]. This suggests that the bilayer, when in the liquid state, is more subject to deformation than when in the gel state, and allows a vesicular suspension to penetrate easily through the skin.

Recently, Tabbakhian *et al.* [37] reported that composition and physical states of vesicles represent important parameters for skin deposition and penetration enhancer behavior of niosomes. Vesicular systems are obtained when film hydration is performed using a transition temperature (T_m) above that of the amphiphiles present in the formulations, suggesting that surfactant agents and other components (i.e., cholesterol and/or negatively charged lipids), added during the preparation procedure, are able to modify mean size, distribution and number of lamella in the nonionic surfactant vesicles. Disk-shaped vesicles or polyhedral multilamellar vesicles with a gel-state conformation were obtained by using Brij72® or Brij76® (Fluka, Switzerland) in niosomal suspensions [38-40]. The physical characteristics (multilamellar structure) of niosomes are not modified by adding cholesterol to the formulations.

Different works [30,41] have reported that steroidal compounds are an important additive in the formation of niosomes due to the ability of molecules to assist the gel to liquid state transition of the niosomal system, and to avoid aggregation phenomena permitting a homogeneous size distribution of the colloidal suspension [42].

An important parameter in the preparation of niosomes is the presence of charged lipids in the bilayer. In particular, it is noteworthy that the addition of negatively charged diacetyl phosphate can prevent aggregation events and reduce the mean diameter of colloidal vesicles, probably due to the increasing curvature of the bilayer, a phenomenon caused by electrostatic repulsion between ionic head groups [30].

The influence of lipid components in the physical state of niosomes is very important in drug deposition and permeation behavior through the skin. It has been demonstrated that the amount of drug permeated through the skin after the topical application of a niosomal formulation during a 24 h period is less than that obtained for a hydroalcoholic solution, suggesting that the multilamellar structure of niosomes is probably the limiting factor for drug permeation [37]. Moreover, data obtained in this experimental investigation showed that the amount of drug deposited in the different strata of the skin is less in the case of niosomes prepared using Brij72 and Span 40® (Fluka, Switzerland) than that obtained for Brij97 and Brij76. Differing behavior of surfactant agents in the deposition of finasteride in the skin compartments is probably influenced by the improved delivery of molecules due to the effect of hydrophilic nonionic surfactants. Tabbakhian's experiments show that the flux of drug through the skin is strictly correlated to vesicle composition and the physical states of colloidal suspensions. **Figure 5** shows the permeation rate of finasteride through the skin. In this case, it is noteworthy that the permeation rate of the drug compound for Brij76 and Brij97 niosomes is higher than that for Span40 niosomes. Differences in drug transport of entrapped compounds are influenced by the physical states of colloidal suspensions. In fact, liquid state vesicles (i.e., Brij76, Brij72 and Brij97 niosomes) and gel state vesicles (Span40 niosomes) are able to modulate interaction with lipids in the skin [37]. This effect may be explained if we consider the interaction with the stratum corneum and differences in partitioning of the drug between vesicles and corneocytes [43]. Surfactants in the liquid state are able to permeate into the intercellular lipid bilayer, thus reducing the packaging structure in the lipids of the stratum corneum and increasing the permeability through this membrane [44]. In contrast, gel-state vesicles are not able to modify the packaging structure of the stratum corneum and a penetration enhancing effect of the vesicle suspension is not obtained [45].

Lipid composition and the phase transition temperature of amphiphiles are able to affect drug deposition in the skin compartments. Colloidal vesicles made using liquid-state amphiphiles (i.e., a Brij76–Brij97 mixture in the lipid composition) produced a high degree of drug deposition in the dorsal skin (4.73 ± 0.33 and 6.12 ± 0.83, respectively); however, niosomes made from Brij52, Brij76, Brij72 and

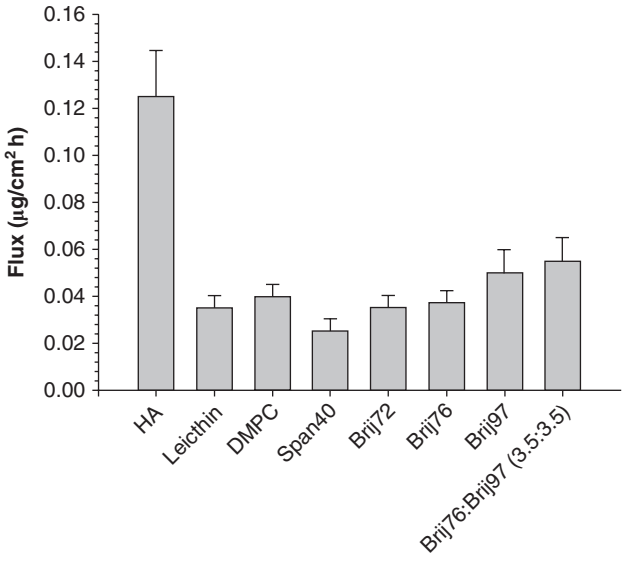

Figure 5. The flux of ^{3}H-finasteride across hamster flank skin from various negatively charged niosomes/liposomes compared with hydroalcoholic drug solution (HA, as control). Liposomes and niosomes were composed of phospholipids (lecithin or DMPC): chol: DCP (8: 2 : 1, m.r.) and surfactant (one or mixture of two): chol : DCP (7: 3:1, m.r), respectively. Each vertical bar and its error bar represent mean ± SD (n = 3).

Reprinted from Tabbakhian M, Tavakoli N, Jaafari MR, Daneshamouz S. Enhancement of follicular delivery of finasteride by liposomes and niosomes. 1. In vitro permeation and in vivo deposition studies using hamster flank and ear models. Int J Pharm 2006;323:1-10, Copyright (2006), with permission from Elsevier.

chol: Cholesterol; DCP: Dicetyl phosphate; DMPC: Dimyristoyl phosphatidylcholine.

Span40 remained on the skin surface in a gel state, thus releasing entrapped molecules. Moreover, niosomes in the gel state may be able to fuse with the lipids in the stratum corneum, thus allowing a lipid exchange that promotes the diffusion of drug compound through the skin [37].

In another study, Fang and coworkers [34] investigated the skin permeation of enoxacin in the case of niosomal suspensions composed of differing percentages of lipids, surfactants and cholesterol. Different compositions of nonionic surfactant vesicles, tested to optimize permeation of the drug through the skin, influenced the percentages of enoxacin encapsulation and the turbidity of formulations. The turbidity of colloidal vesicles has been proposed as an indicator of niosomal sizes. No significant differences in encapsulation efficiency have been distinguished for niosomes prepared using Span40 and Span60, whereas the turbidity of Span40 is higher than that of Span60. The effect of sorbitan monoester on niosomal size has been attributed to the decrease in surface energy together with the increase in the hydrophobicity of the acylic chain. Encapsulation efficiency has also been correlated with the increase in vesicular size and the turbidity of the suspension. Neither Span40 nor Span60 niosomes show any modification in terms of encapsulation efficiency or turbidity after 48 h of incubation; this effect allows us to obtain a colloidal suspension that remains stable during storage time. Stable niosomal formulations were tested as transdermal devices in a nude mouse skin model. Free and niosome-encapsulated enoxacin which permeated across nude mouse skin shows that Span40 and Span60 niosomes facilitated the skin permeation of the drug compound through the stratum corneum with respect to un-entrapped enoxacin or liposomal formulations. Moreover, surfactants acted as permeation enhancers and promoted the diffusion of drug through the skin.

The effect of permeation enhancers on colloidal devices can also be elicited with the fusion of nonionic surfactant vesicles to the interfacing of the stratum corneum. In fact, this mechanism allowed enoxacin to accumulate in the bilayer, thus generating an improvement in the thermodynamic activity of the drug compound in the upper layer of the stratum corneum. The permeation enhancing effect of surfactants becomes more evident when intact skin is pretreated with niosomes. A further increase in the amount of enoxacin permeated across the stratum corneum, as well as greater drug deposition, is obtained when the skin surface of a nude mouse is preventively treated with Span60 niosomes. The cumulative amount of enoxacin in the different skin compartments is higher than that obtained for nontreated skin or free drug. These results suggest that the nonionic surfactant contained in the formulation is able to reduce the barrier effect of the stratum corneum, thus promoting the permeation of molecules through the skin.

Another important parameter in the case of transdermal drug delivery of vesicle devices is the elasticity of the bilayer. The penetration of the lipid vesicles through the skin is a function of carrier membrane deformability.

The accumulation and diffusion of niosomes through the skin is influenced by experimental conditions. The transdermal delivery of nonionic surfactant vesicles/encapsulated tretinoin highlights the fact that the degree of permeation of drug compound through all skin layers is increased when occlusive conditions are applied, whereas nonocclusive conditions do not promote the improvement of drug flow across the skin [32]. Experimental findings show that skin permeation of the drug compound is influenced by lipid composition, physicochemical properties and the surface charge of the formulation. Results obtained by Manconi *et al.* show that unilamellar vesicles are better carriers than multilamellar vesicles for the delivery of tretinoin through the skin. This effect is probably correlated to the difference in terms of the physical stability and mean sizes of colloidal suspensions after their application on the skin. In fact, the less stable unilamellar vesicles disintegrate after contact with the skin surface and are immediately integrated with lipids, thus promoting a lipid exchange between the colloidal vesicles and the cutaneous barrier, during which the diffusion of drug and fragment production of the formulation may occur [46].

2.3 Ultradeformable liposomes (Transfersomes)

Since the 1990s ultradeformable vesicles have been reported in the literature as transdermal drug delivery systems for

many compounds for skin treatment and subcutaneous structure targeting [47]. The effectiveness of Transfersomes was successfully demonstrated using model drugs, such as corticosteroids [48,49], diclofenac [50], Transfenac® (IDEA AG) and compounds having high molecular weights, such as insulin [51-53].

These deformable vesicles are a novel type of liquid state vesicles. Cevc *et al.* introduced the first generation of deformable vesicles, also referred to as Transfersomes, consisting of phospholipids and an edge activator [52,54,55]. An edge activator is often a single chain surfactant able to destabilize the lipid bilayer of the vesicles and increase the deformability of the bilayer by lowering its interfacial tension. The physicochemical characteristics of the vesicles and also the mode of application play a crucial role in vesicle–skin interaction. Vesicles can be applied occlusively (covered by a patch to avoid water evaporation) or nonocclusively (exposed to the air, which results in evaporation of water). The difference in skin interaction between occlusive and nonocclusive application is very important for deformable vesicles. It has been suggested that the transport of Transfersomes is driven by the osmotic gradient across the skin [56,57]. Occlusion would eliminate this osmotic gradient and would therefore be detrimental to the action of the deformable vesicles [58-60]. El-Maghraby and coworkers [61,62], in particular, validated this theory by comparing the skin permeation of estradiol and 5-fluorouracil encapsulated in ultradeformable and traditional liposomes after occlusive and nonocclusive application.

Furthermore, it was suggested that Transfersomes could be used for noninvasive transdermal immunization [58-60]. When Transfersomes labeled with a radioactive marker were applied onto the skin, radioactivity was observed in the liver. This indicates the presence of radioactive particles in the systemic blood circulation, which suggests that the vesicles permeate across the skin. Most of these studies have been carried out *in vivo* in mice. Other investigators have confirmed that deformable vesicles were more effective compared with rigid vesicles for trans-dermal delivery [63].

Other authors studied the skin fluxes of dipotassium glycyrrhizinate and found that they were below the detection limit, whereas skin deposition increased 4.5-fold in comparison to an aqueous control [64]. However, the vesicle composition and sizes used are different from those used by Cevc and El Maghraby. In fact, the limited partitioning into the acceptor phase indicates that the ultradeformable vesicles are not carrying the encapsulated drug into the acceptor phase.

Deformable surfactant-based vesicles were shown to be more effective than rigid vesicles in enhancing the penetration of ^3H_2O across hairless mouse skin *in vitro* [65] and pergolide and rotigotine [66,67] across human skin *in vitro*. The results of pergolide and rotigotine as model drugs have suggested that a penetration-enhancing effect is not the main or the only mechanism of action of the deformable nonionic surfactant vesicles, but that these vesicles act as drug carrier systems. In 2004, Honeywell-Nguyen *et al.* [63] suggested that elastic vesicle material can rapidly (within 1 h) enter the stratum corneum and can reach almost as deep as the stratum corneum–viable epidermal junction. Furthermore, elastic vesicles were more effective compared with rigid vesicles in the enhancement of the transport of ketorolac into the stratum corneum. In 2005, the same research group [68] described elastic vesicles as a tool for dermal and transdermal delivery in comparison with physical methodologies such as electroporation ionthophoresis and microneedles. The mechanism which activates the elastic vesicles is most likely to be that of a drug carrier system.

Recently three *in vivo* studies were performed with human volunteers. In two studies, tape stripping (a method to remove sequential stratum corneum cell layers) was combined with electron microscopy to provide information on the permeation of intact deformable vesicles into the stratum corneum. The results were very remarkable and clearly indicated that the deformable nonionic surfactant vesicles partition with great speed into the deeper layers in the stratum corneum within 1 h. Vesicles were visualized in channel-like structures in the intercellular regions, the size and appearance of which were similar to the channel network visualized after a model lipophilic fluorescent label linked to deformable nonionic surfactant vesicles were applied [69]. The evidence that deformable nonionic surfactant vesicles show fast partitioning into the stratum corneum, both model compounds and deformable nonionic surfactant vesicles follow the same route through human stratum corneum, and no other abnormalities were found in the intercellular lipid lamellae demonstrate that these deformable vesicles do not act as penetration enhancers but more probably act as drug carrier systems. When the application was changed from nonocclusive to occlusive, the presence of lipid plaques was frequently observed, suggesting that occlusion impairs the transport of intact deformable vesicles into the skin.

In another *in vivo* study, tape stripping was used in combination with Fourier transformed infrared spectroscopy, which allows quantitative evaluation of the distribution profile of lipid material and the drug compound. This study confirmed that deformable nonionic surfactant vesicle material rapidly enters the deeper layers of the stratum corneum and could reach layers almost as deep as the stratum corneum–viable epidermal junction within 1 h. The distribution profiles of the vesicle material and the drug suggest that the model drug was associated with vesicle material in the upper and central layers of the stratum corneum, but was not associated with vesicle material in the lowest layers of the stratum corneum and hence must have been partly released from these vesicles. This indicates that deformable nonionic surfactant vesicles mainly remain in the stratum corneum and that drug molecules are released from these vesicles with subsequent transport of free drug molecules into the viable skin layers [70].

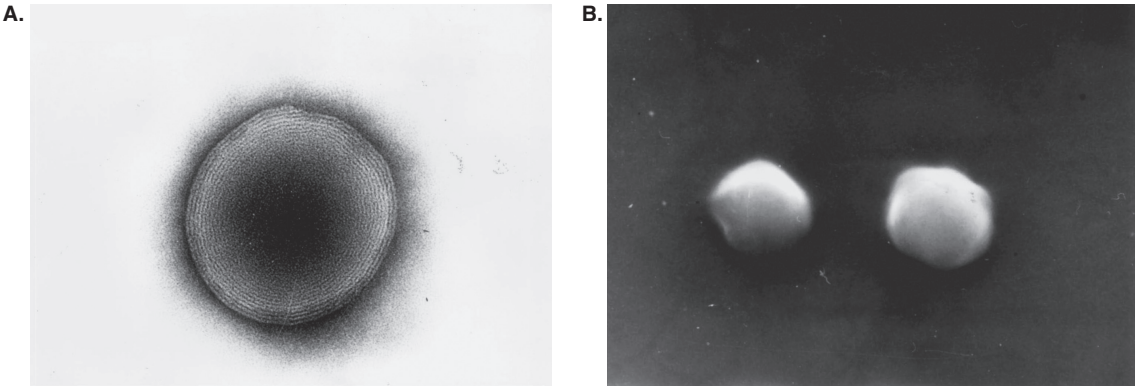

Figure 6. Visualization of ethosomal vesicles. A. Transmission electron microscopy (magnification × 315,000) and **B.** scanning electron microscopy (magnification × 100,000) of ethosomal vesicles composed of 2% phospholipids, 30% ethanol and water.
Reprinted from Touitou E, Dayan N, Bergelson L, et al. Ethosomes - novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Control Release 2000;65:403-18, Copyright (2000), with permission from Elsevier.

2.4 Ethosomes

Ethosomes are novel vesicular carriers developed by Touitou and coworkers [71,72], made up of phospholipids, ethanol and water. Unlike liposomes, a classic and more well known vesicular system made up of the same components without ethanol, ethosomes showed a better enhancer penetration though the stratum corneum barrier, high encapsulation efficiency for both hydrophobic and hydrophilic drugs and showed more fluidity, malleability and elasticity, important characteristics of this carrier which help promote the penetration of the drug compound across the stratum corneum barrier [73]. Studies carried out by transmission electron microscopy and scanning electron microscopy have confirmed that ethosomes have a closed spherical shape and that they appear as homogeneous multilamellar vesicles (**Figure 6**) [74].

Other works have demonstrated that ethanol is the outstanding component which confers important characteristics in terms of drug penetration to the deeper skin layers [71]. Moreover, numerous experimental works have demonstrated that the increase in ethanol percentage up to 45% gradually leads to the smaller vesicles sizes [75].

Ethanolic solvent alone is a well known permeation enhancer. However, studies that have compared the permeation of drug compounds between ethosomal systems and hydroethanolic, ethanol and ethanolic–phospholipid solutions showed that the ethosomes exert great permeation enhancement. The composition of the ethosomal formulations and the different preparation processes can favor the development of a multilamellar or unilamellar structure. The presence of a multilamellar structure increases the encapsulation efficiency of lipophilic drugs, such as testosterone and minoxidil [71].

Ethosomes show a good permeation across the skin, taking advantage of the passive diffusion process. The presence of ethanol, besides giving fluidity, malleability and elasticity to the ethosomal bilayer, confers a soft structure and, interacting with the stratum corneum phospholipids, induces a temporary disorganization of their alchilic chains [76,77]. This phenomenon facilitates the crossing of the vesicles through the stratum corneum and, by the passive diffusion process, the active compounds loaded inside the carriers can reach the deeper skin layer where they can explicate their pharmacological action.

In a recent experiment [78], an ethosomal system was used to load acyclovir (ACV), a synthetic acyclic nucleoside analog, with the aim of treating *Herpes labialis* pathologies. In this randomized clinical study, a commercial acyclovir cream (Zovirax®, GlaxoSmithKline S.p.A.), an ethosomal formulation and a solution of the free drug were compared at the same concentrations. The results showed that the ethosomal–acyclovir had the better clinical efficacy with respect to Zovirax cream. In fact, it shortened the period of time to crust formation and to the loss of the crust compared with conventional Zovirax cream.

In another experiment, the enhanced skin delivery of ethosomes was described. The ethosomal system loaded with testosterone and minoxidil was compared with ethanolic, hydroethanolic and phospholipid ethanolic solution in *in vitro* and *in vivo* experiments. An encapsulation study showed that the carrier is able to load both hydrophilic and hydrophobic compounds but a hydrophilic drug was less efficient in the entrapment phase than a hydrophobic drug and this phenomenon was demonstrated using rodamine and calceine (hydrophobic and hydrophilic probes, respectively) observed through calorimetry and fluorescence measurements. Ethosomes were able to load a large quantity of testosterone and minoxidil (90 and 83%, respectively) and to enhance the penetration of a relatively large amount of drug compound into the skin. Data show that an ethosomal carrier effectively delivers components deeply into and through the skin. In fact, the permeation profile of ethosomal–testosterone and commercial Testoderm® (Alza, Palo Alto, CA, USA) was evaluated using Franz diffusion cells equipped with rabbit pinna skin. The results of this experiment show that after

5 days the area under the concentration time curve of the colloidal formulation is about 125% greater than that of commercial cream [71].

Trihexyphenidyl HCl (THP), a cationic anti-M1 muscarinic drug used for the Parkinsonian syndrome and drug of choice for dystonia therapy, was loaded into an ethosomal system in another important experiment. Normally THP is administrated orally and shows various adverse effects that could be avoided by loading it into an ethosomal carrier. The experiment showed that the use of an ethosomal THP system permits constant systemic drug compound levels and avoids peak blood concentration, given a correct administration and good patient compliance. Patient compliance is aided by the fact that normal oral administration of the drug compound is a drawback since a foremost manifestation of Parkinson's syndrome is difficulty in swallowing. To test an ethosomal THP system, it was compared with a liposomal THP system and a hydroethanolic solution (30% w/w ethanol). The results showed that the ethosomal formulation increased the amount of THP permeated into the skin over time compared with the liposomal system and the control solution. Moreover, the ethosomes showed better entrapment efficiency with respect to liposomes. Furthermore, whereas empty liposomes have a slight positive charge, empty ethosomes have a negative charge which increases ethanol concentration. So the encapsulation of THP in this carrier brings about changes in the overall charge, gradually increasing the positive charge as the THP concentration increases [73].

In an additional study, it was demonstrated that ethosomes are ideal vesicular systems for the delivery of molecules with different physicochemical characteristics into and through the skin and into the fibroblast cells when compared with a hydroethanolic solution and classic liposomes. Penetration studies of fluorescent molecules were carried out by CLSM and fluorescent-activated cell sorting (FACS). The ethosomal system reached fluorescence intensity within 10 min and stayed constant for 20 min in contrast with the hydroethanolic control solution and liposomes. The same studies were carried out by FACS and it was demonstrated that only in the cells treated with the ethosomal carrier is there a significant probe penetration. Every probe showed a different amount of distribution in the cells, and the amphipathic probe was present throughout each cell, including the nucleus. Fluorescent lipids were most highly concentrated in the outer membranes of cells and in some of the nuclear organelles. After the treatment of nude mice with ethosomal systems, a difference in fluorescence intensity in the various skin layers was found. The maximum fluorescence intensity of calcein was obtained when the probes were delivered by ethosomes. The intensity remained constant throughout 50 μm of skin depth and finished at 160 μm in contrast with the hydroethanolic solution and liposomes (60 and 80 μm, respectively). In the same study, the use of lipophilic rhodamine red-X 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine triethylammonium salt (RR) gave a different penetration pattern in which fluorescence intensity finished at 260 μm skin depth for the ethosomal formulation, while for liposomes and the hydroethanolic solution it finished at 20 and 40 μm, respectively. Naturally, studies of live/dead viability/cytotoxicity were carried out to demonstrate the cell membrane integrity and whether the probes interacted with live cells. Results from intracellular delivery of RR and [1-palmitoyl-2-[12-7-nitro-2-1,3-benzoxa diazil 1-4 yl amino [dodecanyl] sn-glycero-3]]-phosphatidylcholine indicated a good penetration into the fibroblast and, in particular, tests on D-289 cells showed a nuclear localization. Their highly efficient delivery and nontoxicity make these systems good candidates for delivery of biological and chemical molecules to both skin and cells [79].

Cannabidiol is a potent anti-inflammatory agent used for the treatment of rheumatic diseases. Unfortunately, the oral and intraperitoneal administration of this drug is associated with various drawbacks: low oral bioavailability, extensive first pass hepatic metabolism, instability in the acidic gastric pH and/or low water solubility besides low patient compliance. This is why transdermal administration using a novel dermal drug delivery system, like Ethosome, could be a possible way to reduce its toxicity. CBD ethosomes were prepared containing a eutectic mixture of CBD/PL and EtOH. The eutectic mixture CBD/PL90 was prepared and visualized by CLSM. This formulation was investigated in *in vitro* and *in vivo* experiments using nude mice to study skin accumulation of CBD ethosomes. The results showed an important drug accumulation *in vitro* (49%) in the skin after 24 h whereas during the *in vivo* experiment drug accumulation both in the skin and in the underlying muscle [80] was detected.

In another study, the penetration properties (through skin and cell membranes) of Bacitracin™ (Sigma) loaded in ethosomes were investigated. Bacitracin (Bac) is a polypeptide antibiotic extracted from cultures of *Bacillus subtilis* and *Bacillus licheniformis* which acts against Gram-positive bacteria. It is capable of provoking severe allergic reactions and can have other important adverse effects. An ethosomal system could drive a large amount of active compound to the site of infection normally inaccessible through the dermal route because of the physicochemical characteristics of the drug. In this study, we investigated the intracellular delivery of Bac and fluorescein isothiocyanate bacitracin (FITC-Bac) using ethosomes compared with classic liposomes. Moreover, both the vesicular systems contained RR (a hydrophobic drug, localized in the membrane) with the aim of visualizing the different localizations of RR and FITC-Bac (water soluble, localized in the core of the vesicle) by CLSM. These experiments defined the mechanisms of internalization and intracellular delivery mediated by ethosomes. In fact, CLSM showed that fluorescent phospholipids of ethosomes were located in the cellular membrane, whereas ethosomal FITC-Bac was located inside the cells. The cells appeared

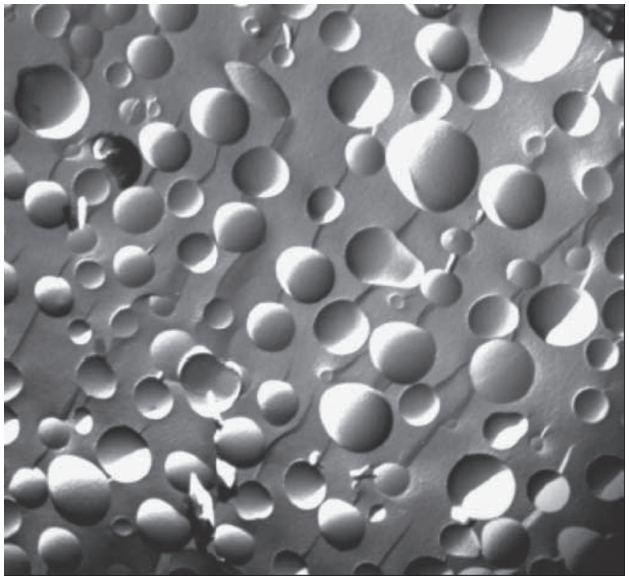

Figure 7. Freeze fracture electron micrographs of ethosomal formulation made up of ethanol (45% v/v) and lecithin (2% w/v).
Reprinted from Paolino D, Lucania G, Mardente D, et al. Ethosomes for skin delivery of ammonium glycyrrhizinate: in vitro percutaneous permeation through human skin and in vivo anti-infl ammatory activity on human volunteers. J Control Release 2005;106:99-110, Copyright (2005), with permission from Elsevier.

highly fluorescent both at 488 nm (FICT-Bac wavelength) and at 523 nm (RR wavelength). The same experiment was carried out with liposomes containing both probes, but a significantly lower fluorescence intensity was observed. These results correspond to those obtained for the intracellular delivery of fluorescent-labeled Bac, which was investigated and evaluated by FACS on dermal fibroblasts of a Swiss albino mouse (3T3). In particular, the different fluorescence intensity demonstrated a significant lower intensity of liposomes with respect to the ethosomal carrier both at 10 and at 60 min. The penetration profile of FITC-Bac ethosomes was investigated using Franz diffusion cells equipped with human cadaver skin which was analyzed by means of CLSM. The results showed that both probes contained in the ethosomal system had a maximal depth in the skin (200 µm) and that the carrier penetrated into the skin together with the drug. The maximal fluorescence intensity was reached delivering probes by ethosomal carriers (about 160 and 90 arbitrary units, respectively), whereas liposomes showed a very low rate of delivery. FITC-Bac ethosomes were also used for skin penetration and distribution *in vivo* to the rat skin and the experiments were monitored by CLMS. The results demonstrated that drug penetration took place through a diffused penetration pathway and a high grade of fluorescence was observed in different depths of skin layers. In contrast, for the hydroethanolic drug solution there was a weak coloring of the upper skin layers and for FITC-Bac-loaded liposomes there was the high-fluorescence intensity associated with hairs and upper skin layers. The results obtained in this study showed the highly efficient delivery system of ethosomal vesicles in the treatment of intracellular infections [74].

In a recent study [75], the potential dermal application of ammonium glycyrrhizinate (AG) (a drug obtained from *Glycyrrhiza glabra* characterized by anti-inflammatory activity) was investigated, loaded both in an ethosomal system and in ethanolic or aqueous solutions for treatment of inflammatory skin diseases. The colloidal carriers were characterized by photon correlation spectroscopy and freeze fracture electron microscopy (**Figure** 7). Studies of percutaneous permeation of AG loaded in ethosomes were carried out using Franz diffusion cells equipped with human stratum corneum and epidermis membranes. The carrier toxicity was evaluated using a noninvasive technique (reflectance visible spectrophotometer) on human volunteers. The same technique was used to evaluate the erythema index (ΔEI) on volunteers treated with methyl nicotinate (a vasodilating agent) to induce erythema on specific arm sites. The volunteers were successively treated with ethosomal-AG, a hydroalcholic solution and an aqueous solution of the drug plus a physiological solution (NaCl 0.9%) as control. The results showed that AG-ethosomes induced a significant reduction in the intensity and the duration of erythema with respect to the other formulations. In particular, the percentage of maximal ΔEI derived from the use of ethosomal-AG 5 h after treatment (29.6%) was considerably lower than that for the ethanolic and aqueous solutions (62.7 and 60.7%, respectively) [75].

2.5 Polymeric particles and solid lipid nanoparticles

Micro and nanoparticles are solid colloidal suspensions in which the mean particle size is > 1 µm or less than 1 µm, respectively. They can be classified morphologically in spheres and capsules. In the first case, the particles are made up of a porous/dense matrix in which the drug can be adsorbed and/or entrapped, while capsule systems are formed by a core (aqueous or lipophilic) containing drugs surrounded by a shell [81].

The use of a large series of polymers is limited by their bioacceptability. In fact, the most widely used polymers for particles are poly(lactic acid) (PLA), poly(glycolic acid), and their copolymers, poly(lactide-co-glycolide) (PLGA) which are known for their biocompatibility (they are accepted by the Food and Drug Administration) [82] while other types of polymers used in the particles' preparation are polyalkylcyanoacrylate, polyvinyl alcohol, poly(ε-caprolactone) and chitosan.

The mechanisms of drug release from nanoparticle colloidal suspensions depend on the characteristics of the colloidal suspension as well as on physicochemical properties of the drug. In particular, the release of a drug may occur by one of the following mechanisms or a combination of more than one of them: drug desorption from the colloidal surface (both for nanospheres and nanocapsules); drug diffusion through the polymeric network of the nanospheres or

through the polymeric shell of nanocapsules; and polymeric matrix erosion of nanoparticles [82].

Different particle structures give origin to different types of drug release. In particular, the compounds loaded in spheres will be released by a zero-order kinetic while the drugs entrapped in the cores of the capsules will be released by a first-order kinetic.

Because of their characteristic properties, polymeric particles were used as innovative delivery systems to enhance percutaneous transport into and across the skin barrier. In particular, the possibility of assuring a sustained release of the active compounds represents an enormous advantage in topical treatment. For example, ACV was encapsulated into PLGA microparticles with the aim of evaluating the amount of antiviral drug accumulated in the different strata of porcine skin *in vitro* with respect to that obtained using an aqueous suspension of the drug. The spheres, having a mean size of about 4 – 5 μm, showed a loading capacity of ~ 50% while the ACV released was ~ 80% during the first hour (burst effect) and ~ 20% in the next 7 days. Moreover, subsequent to topical application, the amount of ACV found in the skin after 24 h was higher with the microparticles than with the control suspension, with a predominance of distribution in the upper layers of the skin (100 – 200 μm depth). This phenomenon can be explained by the occlusive effect of the polymeric carriers which reduces transepidermal water loss and improves the drug penetration into the skin [83]. In another work [84], a different antiviral compound, cidofovir, was encapsulated in PLA/PLGA microparticles which were obtained by means of two different preparation techniques, in particular the water in oil in water (W/O/W) solvent evaporation method and spray drying. Evaluation of the physicochemical properties of the two formulations showed that the particles prepared by the spray-drying method had a smaller diameter than those obtained by the solvent evaporation method and a significantly higher encapsulation efficiency (80 versus 10%, respectively). Also, permeation studies between polymeric microparticles loaded with cidofovir and a solution of the active compound were carried out using porcine skin. The results indicated that the penetration of cidofovir through porcine skin was greater in the case of the drug solution than in the case of the microparticles (5.27 ± 0.31 versus 3.63 ± 0.22 μg/cm^2 h respectively), while the amount of drug compound found in the upper layers of the skin (to a depth of 180 – 270 μm) was greater with microparticles than with the compound solution (occlusive effect), although from 270 to 800 μm there were no significant differences between the two formulations. If it is assumed that the basal epidermis (site of herpes virus lesions) is located at a depth between 120 and 150 μm, it is clear that acyclovir and cidofovir loaded PLA microparticles could represent a new approach for treating these skin pathologies.

Polymeric particles were also used to encapsulate anticancer drugs. For example, 5-fluorouracil, a competitive inhibitor of thymidylate synthetase, was loaded inside poly(butylcyanoacrylate) nanoparticles with the aim of treating premelanoma pathologies such as skin lesions. In this case, the polymerization kinetic of the alkylcyanoacrylates is favored by the basic nature of the 5-fluorouracil saline solution, which makes the formation of particles with a diameter of ~ 120 nm possible, thus entrapping about 70% of the antitumoral compound [85].

More recently, Shim *et al.* [86] evaluated the effect of self-assembled nanoparticles on the skin penetration of minoxidil *in vitro* and *in vivo*. The permeation profile of the aqueous nanoparticle suspension through the abdominal skin of guinea pigs [the suspension being composed of poly(ε-caprolactone)-block-poly(ethylene glycol) (10% w/w), minoxidil (0.5% w/w) and ethanol (2% w/w)] was compared with a liposomal formulation (lecithin/minoxidil/ethanol 4/0.5/10% w/w) and with an ethanolic solution of the drug at the same concentration using Franz-type diffusion cells. Minoxidil-loaded copolymer nanoparticles (mean sizes of ~ 40 nm) showed the best results when compared with the other formulations (23% of the total compound applied was detected in the receptor compartment and 3% of the drug was detected in the skin). Successively, rubrene (5,6,11,12-tetraphenylnaphthacene) was encapsulated into the nanoparticles, which were applied to guinea pigs *in vivo*. After biopsy, the skin samples, analyzed by CFLM, showed that the fluorescent compound was more concentrated along hair follicles or in the tissue near the follicles than at other sites. The studies showed that this type of polymeric nanoparticle mainly penetrates through shunt routes like skin appendages and can deliver minoxidil to skin very effectively. These results were confirmed in recent times by a study of the penetration of 5-fluoresceinated Resomer® (Boehringer Ingelheim Phama Chemicals) RG 50 : 50H nanoparticles (diameter 320 nm) with respect to the same amount of dye in the nonparticle form into the hair follicles of porcine skin *in vitro* [87]. The two formulations were homogeneously massaged into the ear skin and, after biopsy, the histological sections were analyzed using a laser scanning microscope by superposition of the transmission and fluorescent images. As can be seen in **Figure 8**, the particles penetrated much more deeply into the hair follicles than the dye in nonparticle form.

In recent years, nanoparticles have also been used in the vaccination field as nonviral gene delivery systems. For example, Cui and Mumper [88] investigated the topical application of chitosan-based nanoparticles containing plasmid DNA (pDNA) as a potential approach to genetic immunization. They used two types of particles, pDNA-condensed chitosan nanoparticles and pDNA-coated preformed cationic chitosan/carboxymethylcellulose (CMC) nanoparticles. Levels of luciferase expression were quantified 24 h after topical application on the shaved skin of Balb/C mice. In particular, all the selected nanoparticle formulations showed levels of luciferase expression at 24 h to be higher than that of 'naked' plasmid DNA alone, while the IgG titer

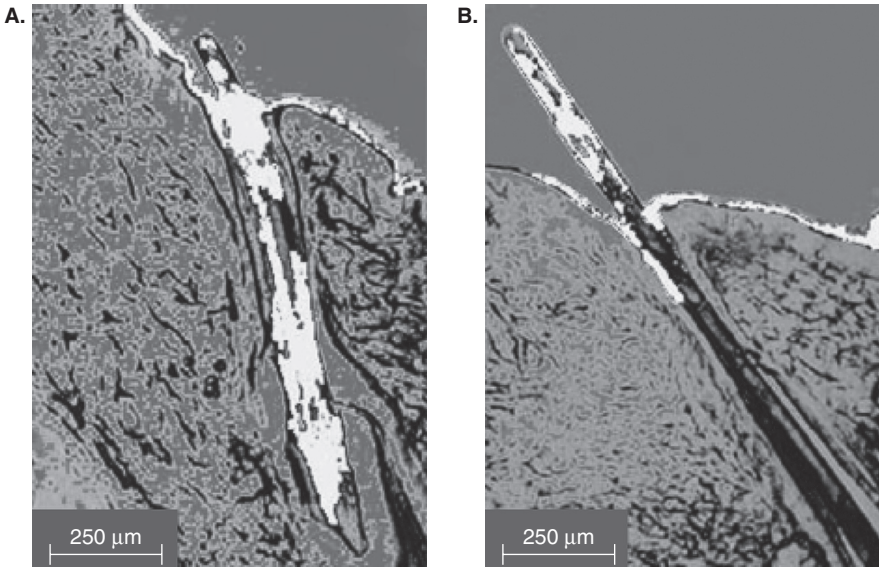

Figure 8. Superposition of a transmission and fluorescent image, demonstrating the *in vitro* penetration of the dye-containing formulation into the hair follicles of porcine skin after application of a massage. A. Dye in particle form. **B.** Dye in non-particle form.
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was up to 32-fold greater when mice were immunized with pDNA coated on chitosan oligomer/CMC nanoparticles (300:100 w/w) with respect to those mice immunized with 'naked' pDNA alone. Moreover, IgG titers of mice treated with this nanoparticle formulation were comparable to those of mice immunized by intramuscular injection of 'naked' pDNA but the polymeric system did not increase the IgG plasmatic concentration. Cutaneous gene therapy was also used by Badea and coworkers [89] to administer IFN-γ gene by means of Gemini nanoparticles in IFN-γ-deficient mice. The particles (NP16) were made up of the gemini cationic surfactant *N,N′-bis*(dimethylhexadecyl)-1,3-propanediammonium dibromide (16-3-16), 1,2 dioleyl-sn-glycero-phosphatidylethanolamine, 1,2 dipalmitoyl-sn-glycero-phosphatidylcholine and diethylene glycol monoethyl ether. The NP16 formulation exhibited a particle population having a mean size of 219 ± 49 nm while the addition of DNA to NP16 resulted in an essentially bimodal distribution for the main scattering population with average sizes of 148 ± 41 and 468 ± 115 nm IFN-γ-deficient mice (0.067 ng/cm^2 of protein expressed) treated with plasmid-NP16 nanoparticles showed that gemini nanoparticles improved the transgenetic expression threefold with respect to a plasmid DNA solution (0.480 versus 0.167 ng/cm^2, respectively).

Solid lipid nanoparticles (SLNs) were developed in the early 1990s as an alternative carrier system to emulsions, liposomes and polymeric nanoparticles. These are nanoparticles with a solid lipid matrix presenting an average diameter in the nanometer range, which protects the incorporated active compounds against chemical degradation and modulates their release (drug mobility is much slower in a solid lipid than in a liquid oil). Normally, SLNs are made up of solid lipids (triglycerides, partial glycerides, fatty acids, steroids and waxes), emulsifiers (poloxamer 188, polysorbate 80, polyvinyl alcohol, sorbitane monopalmitate), and water, all of which are generally recognized as safe substances. The topical application of SLNs favors skin hydration, an effect that is due to their spatial disposition. In particular, the reduced mean sizes of the nanoparticles and the extremely small holes between the nanoparticles result in a uniform, poreless film which is more able to prevent water evaporation with respect to the microparticles [90]. Moreover, a great advantage of SLNs used in topical application is their protective action on the natural lipid element of the skin induced by their reflective properties, a characteristic which is missing in the other formulations, such as nanoemulsions, which are of comparable composition (scattering of UV rays) [91]. This characteristic effect is increased by the low skin permeation profile of SLNs, which is confined to the stratum corneum. Many research groups utilized these specific properties to improve the UV absorbance of numerous active compounds. For example, Jenning *et al.* [92] used SLNs to topically deliver vitamin A, a potential UV absorber, and evaluated the release profile of the active compound compared with that obtained using nanoemulsions. In the first 10 h the release of vitamin A from nanoemulsions was considerably greater than that of SLNs before and after the incorporation of the particles into hydrogels made up of different polymers used to obtain the semisolid preparations for the experiment. Conversely, during the following hours the release rate of the drug compound from SLNs even exceeded the flux of the emulsion. This phenomenon may be explained by the hypothesis that the slow release profile of SLNs (first 6 h) is probably due to the solid matrix,

but after 24 h the aqueous evaporation of the system which has taken place in the meantime transforms them into a semisolid gel which allows the higher diffusion velocity of the active compound compared with the emulsion (which remained in the liquid state). In the case of SLN-loaded hydrogel, water evaporation was reduced probably because components such as glycerol and xanthan gum have water-binding properties. After 6 h in particular, the amount of retinol found in the receptor compartment was less than that obtained from a nanoemulsion-loaded hydrogel [92] while, after 24 h the release profiles were very similar. The use of hydrogel allows the formulation to achieve a sustained drug release over a 24 h period compared with that achieved by a conventional SLN system. The use of hydrogel also improves the stability profile and the skin application properties of this kind of particle [92].

More recently, a new lipophilic sunscreen compound made up of chitin and 3,4,5-trimethoxybenzoyl chloride, 3,4,5-trimethoxybenzoylchitin (TMBC), was loaded into SLNs and its capacity to absorb UVB rays (~ 270 nm) in free form was compared with that of the encapsulated form. SLN-TMBC showed mean sizes of about 300 – 500 nm and improved the UVB absorption property (~ 300 nm) of the active compound. Moreover, it was found that the use of vitamin E in the SLN formulation further improved the absorption of UBV, although the size of the carriers was greater (~ 1 μm) [93].

The goals of assuring sustained release, skin-targeting potential and a reduced systemic absorption of the active compounds led us to utilize SLNs to deliver drug compounds presenting side effects. In particular, betamethasone 17-valerate (BMV), a potent glucocorticoid used in the treatment of atopic eczema, was incorporated into solid lipid nanoparticles to reduce the risk of dermal atrophy. Different types of lipids (Compritol® ATO 888, Monosteol®, Precirol®, Geleol®, Softisan® 601; Gattefossé, Germany) and concentrations of lipophilic phase and surfactants (Poloxamer 188, Polysorbate 80) were used to evaluate the more stable formulations. Two types of preparations showed the best physicochemical characteristics and stability profiles. In particular, mean sizes of SLNs made up of Compritol 12.5%/Poloxamer 188 3% and Precirol 12.5%/Polysorbate 80 3%, both of them containing BMV at 0.1%, were 304 and 214 nm respectively, and their stability was preserved for about 3 months (only the Compritol formulation had a polydispersion index variation from 0.22 to 0.47). Cutaneous penetration experiments were carried out by applying the two SLN formulations and a commercial O/W cream loading BMV at the same concentration on fresh human abdominal or breast skin obtained from females after cosmetic surgery. After 6 h the Compritol SLNs improved the penetration of the active compound into the skin (first two 100 μm layers) fourfold, while Precirol SLNs did so less than twofold with respect to the cream [94].

In another study [95], podophyllotoxin, an active compound which inhibits the growth of epithelial cells infected by human papilloma virus in the epidermis, was loaded into SLN systems to reduce the drug compound's severe side effects resulting from systemic absorption [95]. For this experiment, two types of podophyllotoxin-loaded SLN formulations were prepared: the first (P-SLN) was made up of tripalmitin, soybean lecithin, Poloxamer 188 and water, while the second (T-SLN) was prepared using tripalmitin, Polysorbate 80 and water, with the aim of evaluating the influence of surfactants and other factors on the skin-targeting abilities of SLNs. The formulations, after homogenization (five cycles), showed different size distribution. In particular, the diameter of P-SLNs was ~ 70 nm while that of T-SLNs was ~ 120 nm but having a double distribution population (44 and 190 nm). Moreover, the presence of the active compound did not influence the zeta potential of SLNs, which were -48.36 mV (P-SLN) and -17.4 mV (T-SLN). In order to evaluate the skin-targeting potential of SLNs, studies of *in vitro* permeation through porcine skin were carried out using Franz diffusion cells and, as a reference, an alcoholic tincture of podophyllotoxin (0.15%) was used. After 8 h no traces of podophyllotoxin SLNs were detected in the receptor compartments, while the amount of podophyllotoxin tincture was about 0.052 ± 0.008 μg cm^{-2}/h. Successively, the quantity of podophyllotoxin accumulated in the skin from the three formulations was evaluated, the results being 23.38 ± 0.55 (P-SLN), 6.82 ± 0.34 (T-SLN) and 6.08 ± 0.31 μg (tincture). The results showed that only P-SLNs significantly increased the accumulative amount of podophyllotoxin in the skin (3.84 times over the tincture) and showed good skin targeting, while T-SLNs failed to increase the absorption of podophyllotoxin in the skin, even though it did avoid systemic uptake. Fluorescent microscopy showed that P-SLNs might penetrate into the skin through two pathways, including the stratum corneum and the hair follicle route. Therefore, it was concluded P-SLNs are capable of epidermal targeting and reduce the adverse effects induced by the systemic absorption of podophyllotoxin [96].

To reduce the toxic effects of another drug, triptolide, Mei *et al.* [97] used a solid lipid nanoparticle dispersion. The active compound, a diterpenoid extracted from the vine named *Tripterygium wilfondil*, demonstrated anti-inflammatory, immunosuppressive, antifertility and antineoplastic activities [97] but also gastrointestinal, urogenital, cardiovascular and circulatory side effects plus hypersensitivity of the skin. This research group characterized the triptolide SLN system and evaluated its anti-inflammatory properties with the aim of using the formulation in clinical topical treatments. Different lipids (tristearin gliceride [TSG], stearic acid), emulsifers (lecithin or Poloxamer 188) and polyethylene glycol (400) monosterate (PEG400MS) were used to obtain the SLN formulations, while *in vitro* permeation studies were performed with a Franz diffusion cell equipped with full-thickness abdominal skin excised from rats. SLNs made up of TSG, lecithin and PEG400MS showed the smallest mean sizes (123 nm) and the lowest polydispersion index

(0.19), while the largest particle diameter was obtained with the formulation made up of stearic acid, Poloxamer 188 and PEG400MS (173 nm). The best cumulative amounts of drug were obtained from the TSG-SLN dispersion (19.3 mg/cm^2), while the lowest cumulative amounts of drug compound were obtained from the stearic acid SLN dispersion (11.4 mg/cm^2) after a 6 h analysis. Successively, inflammation and consequent edema were induced by injection of carrageenan in the rat's footpad and the anti-inflammatory effects of triptolide-loaded SLN were tested with respect to a triptolide solution. The results showed that the SLN dispersion with the smallest particle size (TSG-SLN) possessed the strongest anti-inflammatory activity (twofold higher than that of the triptolide solution). The data obtained suggest that these SLN dispersions can serve as efficient promoters for triptolide penetration into the skin [97].

3. Conclusions

Topical application of drugs, using a noninvasive route of permeation, is an interesting field of investigation. Colloidal carriers are an important development in drug delivery. Depending on the target, the choice will be liposomes, ultradeformable vesicles, ethosomes or particulate systems.

4. Expert opinion

Colloidal drug delivery systems play an important role in the field of topical delivery systems. In our opinion all colloidal carriers present notable characteristics when applied topically.

Classical liposomes, especially unilamellar, are able to deliver an active compound through the skin by means of absorption, lipid exchange and fusion, and to increase the amount of permeated drugs compared with conventional formulations. Another important characteristic is related to the thermodynamic state of the liposomal bilayer (influenced by phospholipid and cholesterol percentages). The incorporation of a drug into liposomes in a gel state permits a slower skin-permeation rate than that for vesicles in a fluid state.

Another class of colloidal carrier is niosomes. In our opinion, use of this class of carrier will increase in the near future because of the possibility of selecting characteristics by means of an accurate choice and synthesis of surfactants.

Ultradeformable vesicles are another important class of carrier as the presence of an edge activator (conferring them elasticity and deformability of the bilayer structure) enables them to permeate narrow pores in the skin. It is our opinion that this kind of carrier must be fully explored for the percutaneous administration of drugs. IDEA AG, the biopharmaceutical company, is developing targeted therapeutics based on the novel Transfersome carriers, and is carrying out Phase III trials in Europe for the treatment of peripheral pain with a formulation of ~ 100 mg of ketoprofen in a Transfersome gel.

Ethosomes present very encouraging characteristics and the presence of ethanol, intercalated in lipid bilayers, does not cause any damage to the skin. Novel Therapeutic Technology, Inc., is a biopharmaceutical company that has developed a lot of pharmaceutical formulations based on ethosomes technology, including formulations for the treatment of alopecia, deep skin infection, herpes, hormone deficiencies, inflammation, postoperative nausea, atopic dermatitis and erectile dysfunction. In our opinion, this carrier will be further investigated for the delivery of biological compounds, a field where to date no studies have been performed.

Nanoparticles and solid lipid nanoparticles have been extensively investigated, and they show an important effect of sustained release when applied topically. In our opinion, the principal goal for this kind of carrier, which may be exploited in the future, is skin targeting to reduce systemic absorption and, consequently, the adverse effects induced by the systemic absorption for some substances. An interesting topic of research for the future would be the delivery of biological drugs.

The future will see a combination of these carriers and the creation of mixed carriers to exploit several positive aspects of each carrier and to enable their characteristics to be enhanced, especially for the delivery of biological drugs through the skin.

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Declaration of interest

The authors declare that they have no conflicts of interests concerning the preparation of this manuscript.

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