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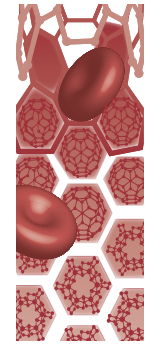

Lipid nanoparticles for the topical delivery of retinoids and derivatives

Retinoids are lipophilic compounds that are highly used in cosmetics/therapeutics for skin disorders. Conventional formulations are limited by poor water solubility, high chemical/photochemical instability and the irritation of retinoids. Interestingly, lipid nanoparticles enable the administration of retinoids in aqueous media, providing drug stabilization and controlled release. Recently, it has been demonstrated that retinoids in solid lipid nanoparticles, nanostructured lipid carriers, nanoemulsions and nanocapsules can decrease degradation, improve targeting and enhance efficacy for the treatment of skin disorders. This article focuses on the formulation, fabrication, characterization and *in vitro*/*in vivo* evaluation of solid lipid nanoparticles, nanostructured lipid carriers, nanoemulsions and nanocapsules loaded with retinoids for skin administration. Furthermore, the incorporation of these lipid nanoparticles into secondary vehicles is discussed.

Keywords: hydrogels • lipid nanosystems • nanocapsules • nanoemulsions • nanostructured lipid carriers • retinoids • skin delivery • solid lipid nanoparticles • topical delivery

Javier O Morales[1,2], Karina Valdés[1], Javier Morales[1] & Felipe Oyarzun-Ampuero*[,1,2]

[1]Department of Sciences & Pharmaceutical Technologies, University of Chile, Santos Dumont 964, Santiago, Chile

[2]Advanced Center for Chronic Diseases (ACCDiS), Santos Dumont 964, Santiago, Chile

*Author for correspondence:
Tel.: +56 229 781 616
foyarzuna@ciq.uchile.cl

Lipid nanoparticles, retinoids & skin delivery

Lipophilic active compounds are commonly used in the treatment of many skin disorders. Considering the physicochemical characteristics of these molecules, an adequate formulation strategy for topical cosmetics/medications plays a key role in product development. An adequate topical formulation should promote the drug effect at the target site, protect labile actives (e.g., from oxidation and ultraviolet irradiation, among others), and prevent the permeation of these molecules to systemic circulation through the skin layers [1–3].

Retinoids are poorly water-soluble drugs that are chemically related to vitamin A. Retinoids can increase skin elasticity, decrease skin roughness and prevent the peroxidation of skin lipids [4]. They work by exfoliating superficial layers of skin, thus fastening cell turnover, enhancing the appearance of skin. Topical retinoids act as antioxidants, preventing tissue atrophy and the loss of collagen that is generally a result of aging [5]. Retinoids also help to restore normal skin and reduce keratoses in sun-damaged skin [6]. Finally, retinoids show antimicrobial activity against the bacteria that are typically involved in acne [7,8]. Despite the variety of beneficial effects of retinoids in the skin, the development of topical systems containing these molecules present limitations such as poor water solubility, high chemical instability and photoinstability and potential irritation upon administration [9,10].

Nanocarriers applied to skin may alter the flux, provide drug deposition and localization and even selectively permeabilize the stratum corneum [11]. Thus, nanosystems may enable the consecutive and long-term administration of lipophilic drugs to the target tissue and improve their pharmacokinetics by optimizing the necessary dose for treatment. Retinoid-loaded nanostructured systems have decreased the adverse effects of these molecules and protected them against degradation [12–16]. Examples of nanocarrier-

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based lipid core systems containing retinoids are solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), nanoemulsions (NEs) and nanocapsules (NCs), among others (Figure 1). Ourique *et al.* have shown that the incorporation of retinoids in NEs and NCs decreased their photodegradation [12]. Shah *et al.* incorporated retinoids in SLNs and also noted a significant increase in their photostability in comparison with the free form of the retinoids [13]. Finally, authors have demonstrated that the incorporation of retinoids into SLNs [14–15,17], NLCs [14] and NEs [16] significantly improves their effects for the treatment of acne and psoriasis compared with commercial formulations [18].

The present article aims to provide information regarding several retinoids included in selected nanoparticles containing a lipid core (i.e., SLNs, NLCs, NEs and NCs) for skin administration. The article covers the relevant literature, with a special emphasis on formulation, fabrication and characterization. In addition, aspects related to their incorpo-

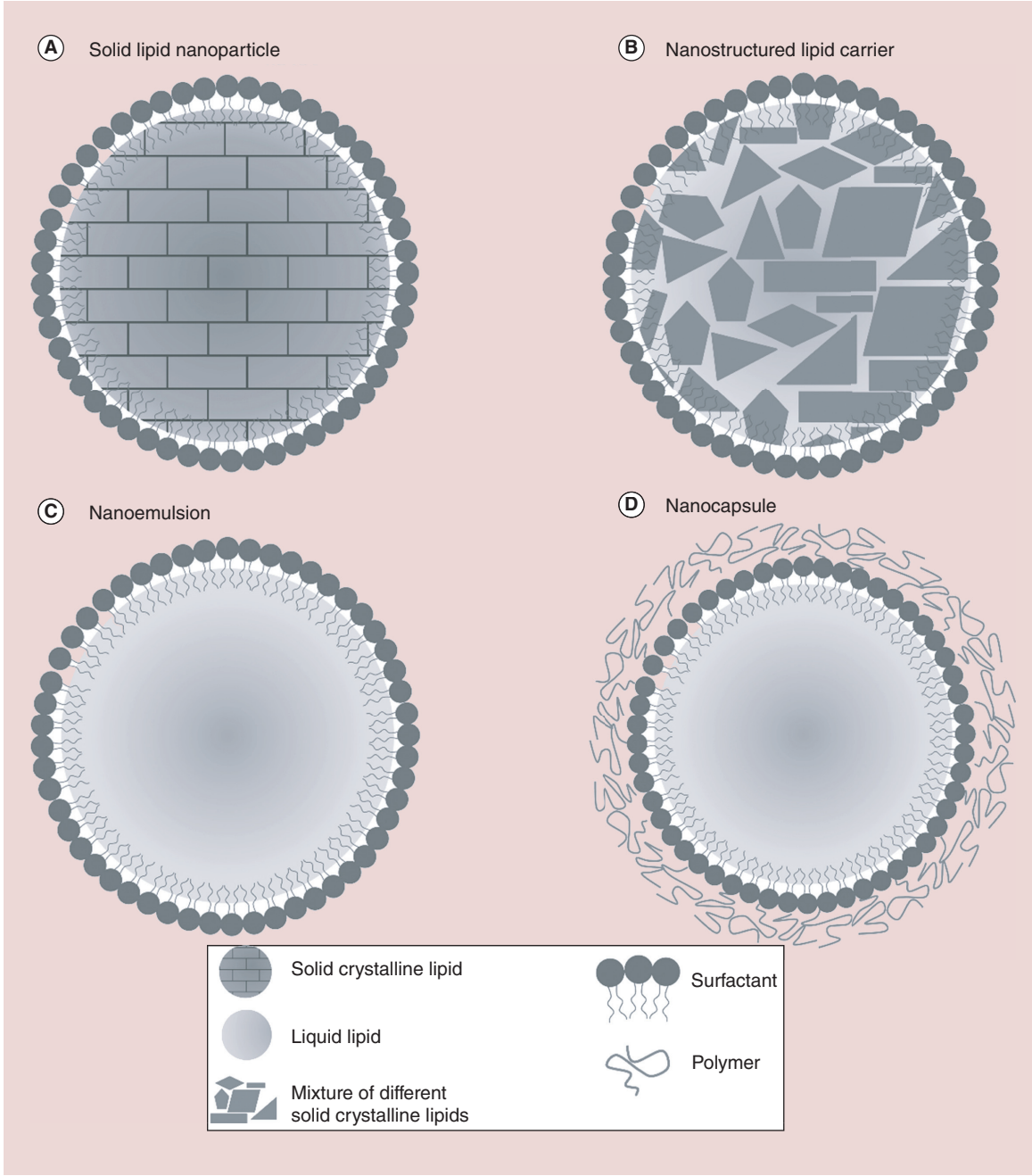

Figure 1. Various types of lipid nanoparticles have been described in the literature, including solid lipid nanoparticles, nanostructured lipid carriers, nanoemulsions and nanocapsules.

ration into semisolid vehicles and *in vitro* and *in vivo* evaluation will be highlighted. This article will focus on the aforementioned lipid-core nanoparticles. For other lipid-based systems containing retinoids, such as liposomes [19–21], niosomes [22–24], mixed micelles [25], nano-lipoidal carriers [26] and lipid–drug conjugates [27], among others [28], the reader is directed to specific publications. In order to identify research within the scope of this article, PubMed and the Web of Science have been used. The keywords used for the search include: retinoids, SLNs, NLCs, NEs, NCs, lipid nanosystems and skin delivery. The date of the last search was 8 July 2014.

Lipid nanoparticles loaded with retinoids

Solid lipid nanoparticles

SLNs overcome a number of the disadvantages of other systems, such as polymeric nanoparticles, liposomes and emulsions [29]. For example, the mobility of the incorporated drugs is relatively high for emulsions and liposomes due to their fluid nature. By contrast, the solid core of SLNs has the ability to reduce the mobility of incorporated drugs, thereby preventing and/or controlling the leakage of encapsulated drug molecules during storage (Figure 1) [30].

In general, SLNs are composed of 0.1–30% (w/w) solid lipids (e.g., cetylpalmitate, Compritol® 888 [Gattefosse, Lyon, France], glycerol monostearate, glycerol palmitostearate, palmitic acid, stearic acid, tripalmitin and tristearin, among others) dispersed in an aqueous medium and, if necessary, stabilized with surfactants (e.g., poloxamer 188, polysorbate 20, polysorbate 80, polyvinyl alcohol, sodium glycocholate, sorbitan trioleate and soybean derivates, among others). The mean particle size of SLNs is from approximately 40 to 1000 nm [31].

NLCs are the so-called second generation of SLNs. In this case, particles are produced using blends of solid and liquid lipids (e.g., oleic acid, Miglyol® [Cremer Oleo Divisions, Hamburg, Germany] and castor oil, among others). Due to the presence of oil in these mixtures, a melting point depression is observed in comparison with the pure solid lipid; however, the blends obtained are solid at body temperature [32]. The incorporation of liquid lipids to solid lipids leads to massive crystal order disturbance. The resulting matrix shows great imperfections in the crystal lattice and leaves enough space to accommodate drug molecules, leading to improved drug loading capacity, preventing its leakage and giving more flexibility for the modulation of drug release (Figure 1) [33,34].

Various fabrication methods for these systems have been described, including high-pressure homogenization (HPH) [35–37], microemulsification [38–40], emulsification–solvent evaporation [41–43], emulsification–solvent diffusion [44–46], solvent injection [47–49], phase inversion [50,51], multiple emulsification [52–54], ultrasonication [55–57] and membrane contactors (Figure 2) [58,59].

HPH can be performed by either the hot or cold method. In the hot homogenization method, the lipid melt containing the active compound is dispersed in a hot surfactant solution of the same temperature (5–10°C above the melting point of the solid lipid or lipid blend) by high-speed stirring. The obtained pre-emulsion is then passed through a high-pressure homogenizer at the same temperature. In the cold homogenization method, the active-containing molten lipid is cooled. After solidification, the mass is crushed and ground to obtain lipid microparticles. The lipid microparticles are then dispersed in a cold surfactant solution, yielding a cold presuspension. Finally, this suspension is passed through a high-pressure homogenizer at room temperature.

Due to the relatively short exposure times to elevated temperatures, homogenization can be used to formulate even heat-sensitive drugs, making this technique the most frequently used. Cold homogenization is recommended for extremely heat-sensitive molecules and hydrophilic molecules, which might partition from the liquid lipid phase to the water phase during hot homogenization.

Several studies have reported the incorporation of retinoids into these lipid nanoparticles [13,33,60–69]. In these works, the incorporation and stabilization of retinoids in SLNs and NLCs was investigated using different lipids as matrix materials and different surfactants and surfactant mixtures. In general, it has been identified that NLCs could improve upon some limitations of SLNs, such as the relatively low drug payload and drug expulsion during storage [36,70]. Unlike the crystalline structure of SLNs, NLCs elicit imperfections in their lipid core matrix, which allows for a higher payload with less drug expulsion [71,72].

In early investigations, Jenning and Gohla tested the inclusion of different retinoids (vitamin A, retinyl palmitate or tretinoin) into nanoparticles comprising Compritol 888 and a mixture of mono-, di- and triglycerides of behenic acid [33]. All of these experiments showed very low drug association efficiency that was improved by the incorporation of small amounts of oils. In a similar strategy, Agrawal *et al.* formulated and characterized acitretin-loaded NLCs in terms of their *in vitro* drug release and clinically evaluated the role of the formulation for the topical treatment of psoriasis [60]. Acitretin or 13-*cis*–*trans* retinoic acid, a metabolite of vitamin A, has physiological effects such as regulation of epithelial cell growth and differentia-

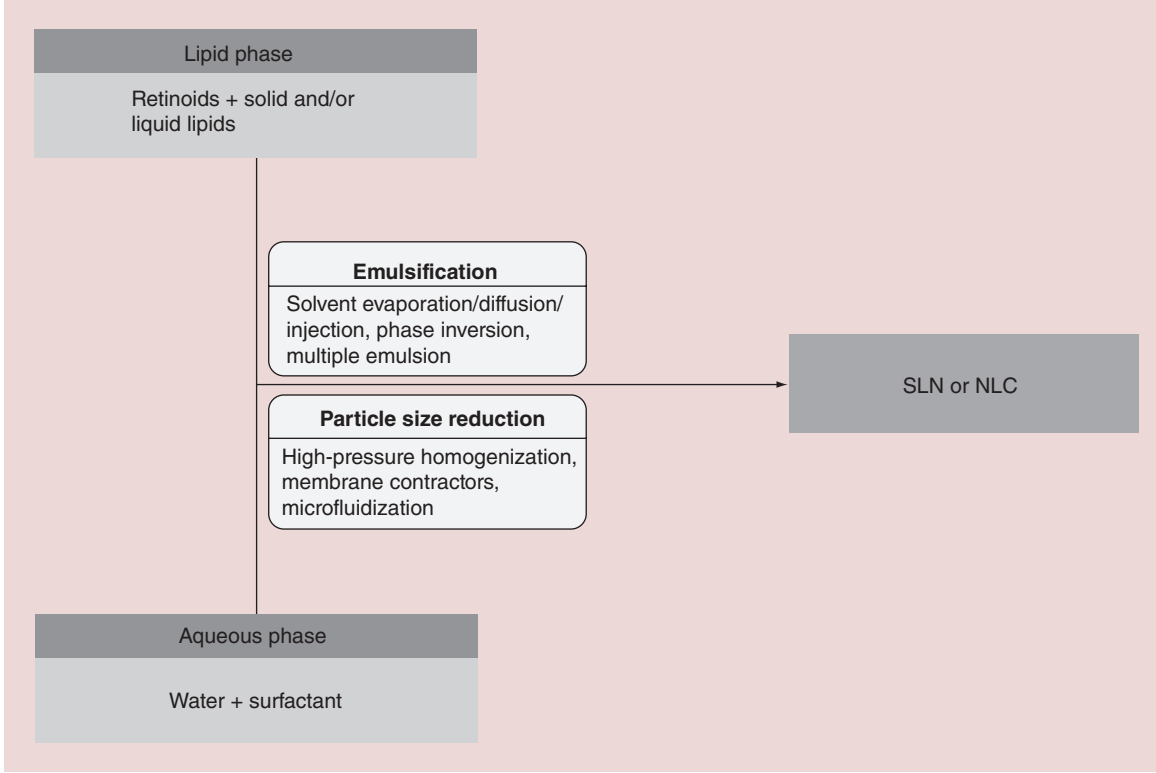

Figure 2. Various fabrication methods for obtaining solid lipid nanoparticles and nanostructured lipid carriers.
NLC: Nanostructured lipid carrier; SLN: Solid lipid nanoparticle.

tion, sebum production and collagen synthesis [73]. In this work, different solid lipids (Compritol 888, Precirol® ATO5 [Gattefosse], Dynasan® 114 [Cremer Oleo Divisions] and glyceryl monosterate) and liquid lipids (oleic acid, Labrafac® Lipo WL 1349 [Gattefosse], Labrafil® M1944 CS [Gattefosse] and Capmul® MCM [Abitec, OH, USA]) were evaluated in order to optimize acitretin loading and release. Interestingly, the particle size of the developed NLCs was inversely proportional to the oleic acid content, with no effect on the ζ-potential value [60].

Lim and Kim have investigated the formulation of all-*trans* retinoic acid (ATRA)-loaded SLNs using tricaprin as the solid lipid as means to improve its water suspendability and the stability of formulations [66]. The addition of polysorbate 80 and egg-phosphatidylcholine resulted in the reduction of particle sizes of SLNs. The ζ-potentials of SLNs were also affected by the combination ratio of egg-phosphatidylcholine and polysorbate 80. Finally, the loading concentration of ATRA linearly increased according to the increase in the initial concentration of ATRA.

Castro *et al.* have investigated the influence of ion pairing between ATRA and stearylamine on the drug encapsulation efficiency (EE) and stability of SLNs prepared with Compritol 888, polyoxyl 20 cetyl ether (surfactant) and cholesterol as cosurfactants [74]. The mean particle size of ATRA-loaded SLNs drastically decreased when the surfactant concentration increased. On the other hand, the EE was significantly improved when the surfactant concentration (or surfactant:lipid ratio) increased. Interestingly, the EE for ATRA was also improved by increasing cholesterol, while the size diminished.

Nanoemulsions

There has been increasing interest over recent years in the use of oil/water NEs to modify drug permeation through the skin [75–79]. NEs are colloidal oil droplets dispersed in an immiscible aqueous medium. The lipid phase could be composed of natural or synthetic oils (e.g., Witepsol® [Cremer Oleo Divisions], Myritol® [BASF, Ludwigshafen, Germany], isopropyl myristate and Miglyol, among others) and surfactants (e.g., polysorbate, Gelucire® [Gattefosse] and PEG, among others). The aqueous phase can be composed of water and cosurfactants (e.g., glycerin and ethylene glycol, among others) if needed. Depending on the elaboration method and drug characteristics, organic solvents could initially be added and evaporated in a further stage (e.g., acetone and ethanol, among others) [75,80].

The methods used to produce NEs can be divided into high- and low-energy processes (Figure 3). High-energy methods use intense mechanical forces to

break up macroscopic phases or droplets into smaller structures and typically involve the use of mechanical devices such as high-shear stirrers, HPH, colloid mills, ultrasonic homogenizers and membrane homogenizers [81,82]. In an example of a HPH method, Benzaria *et al.* have reported the incorporation of retinyl-acetate into peanut oil and stabilized with whey protein [83]. In a first stage, all the components were mixed to obtain a coarse emulsion. Then, the mixture was cycled twice into an ultra-high-pressure homogenizer (200 MPa), obtaining a NE with an average size of 138 nm and a ζ-potential of -24.3 mV. Similarly, Hwang *et al.* developed NEs based on soybean oil and phospholipids and loaded them with ATRA [84]. Here, the coarse emulsion was ultrahomogenized for eight cycles at 150 MPa, reducing the droplet size to approximately 200–400 nm with a ζ-potential of approximately -23 mV. They found that the mean particle diameter had a tendency to increase with the increase in the chain length of the fatty acid in the phospholipids.

Low-energy methods rely on the spontaneous formation of NEs under specific compositions or environmental conditions as a result of changes in interfacial properties. NEs are formed by the rapid diffusion of surfactant and/or solvent molecules from the dispersed phase to the continuous phase without involving a change in the spontaneous curvature of the surfactant [85,86]. In one of the earliest reports, Taha *et al.* described the incorporation of ATRA into NEs [87]. Here, by mixing soybean oil, polyoxyl 35 castor oil and Capmul under magnetic stirring, NEs with narrow size distributions were achieved (51–103 nm). Another interesting work has been reported by Khanna *et al.*, in which NEs were obtained by the vigorous vortex stirring of isopropyl myristate, lecithin, polysorbate and ethanol [88]. The average sizes of these NEs were approximately 110 nm and they were focused on the topical delivery of tretinoin. In another investigation, Moghimipour *et al.* developed NEs loaded with vitamin A, simply by mixing and agitating adequate amounts of surfactants (polysorbate 80 and Labrasol® [Gattefosse]), cosurfactant (propyleneglycol) and an oil phase (isopropyl myristate – Transcutol® P [Gattefosse]) [89]. The resulting NEs had sizes in the range of 14–60 nm and ζ-potentials ranging from -0.07 to -6.41 mV. Similarly, Dizaj studied the use of sunflower oil, polysorbate 80, vitamin A palmitate and different cosurfactants in order to obtain vitamin A-loaded NEs by stirring [90]. In this case, the obtained NEs averaged from 20 to 400 nm in size. With the aim of developing a formulation of adapalene for transfollicular delivery, Bhatia *et al.* elaborated NEs using oleic acid as an oil phase, Tween® 20 (Croda International PLC, NJ, USA) as a surfactant and Transcutol as a cosurfactant

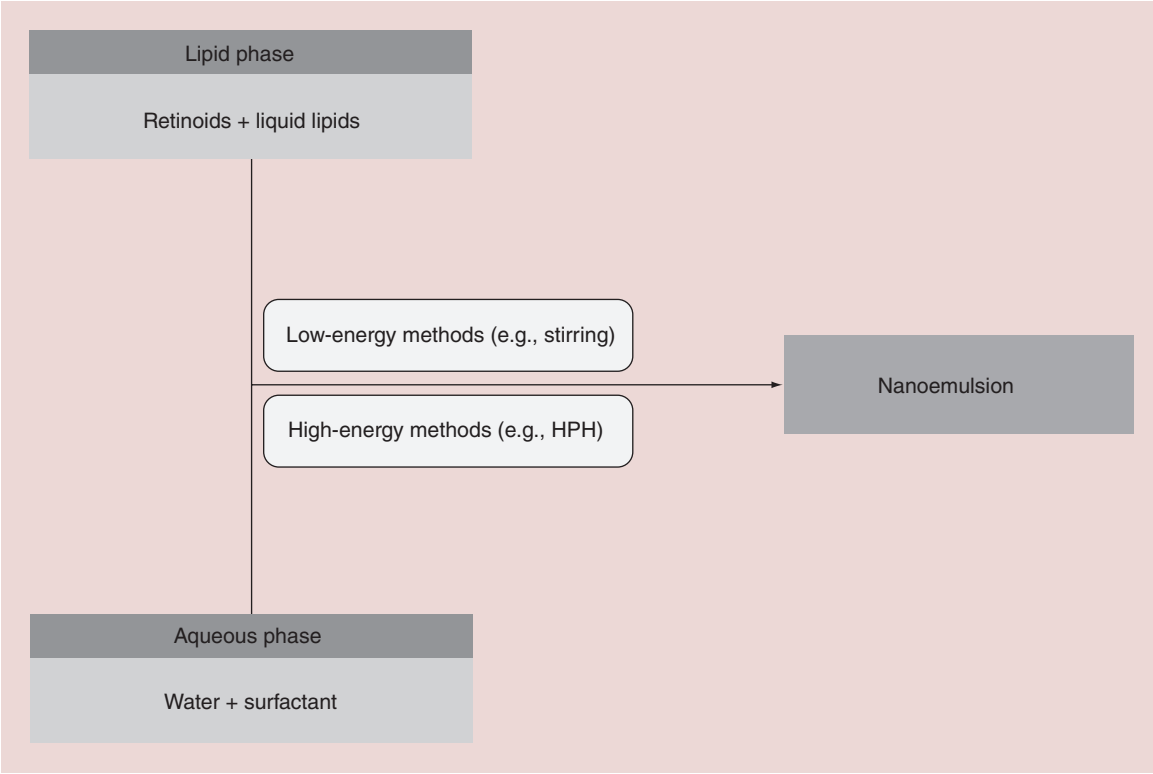

Figure 3. Low- and high-energy methods for obtaining nanoemulsions.
HPH: High-pressure homogenization.

by vigorous vortex stirring [91]. NEs obtained with this formulation had size distributions ranging between 118 and 181 nm. In addition, Oliveira *et al.* developed hydrogel-thickened NEs with tea tree essential oil (*Melaleuca alternifolia*) and retinyl palmitate to be used as a drug delivery system for topical application [92]. Sorbitan monoestearte, PEG-40 hydrogenated castor oil and vitamin A were mixed as the oil phase, followed by water phase addition. Under agitation at 600 rpm, NEs were produced by phase inversion and particle sizes ranged between 86 and 96 nm.

Nanocapsules

NCs have a vesicular organization in which an internal oily reservoir is surrounded by a polymeric coating (Figure 1) [93–95]. The reservoir could be composed of saturated or nonsaturated natural or synthetic oils (e.g., Miglyol, Labrafac, tricaprylin, ethyl laureate, ethyl oleate, corn oil, sunflower oil, sesame oil and soybean oil, among others). This core offers the possibility of great loadings of lipophilic drugs while protecting the drug from the physiological environment. The polymeric coating could be selected from natural or synthetic polymers (e.g., polyalkylcyanoacrylate, polymethacrylate derivatives, polyesters, chitosan and PEG, among others) and the interaction with the reservoir could be mediated by hydrophobic and/or ionic interactions [96,97].

Various methods have been used for the fabrication of retinoid-loaded NCs, including hot HPH [8], nanoprecipitation [12,98–101] and by the simple addition of an organic phase into an aqueous phase containing polyelectrolytes (Figure 4) [102].

Ridolfi *et al.* have reported on the fabrication of NCs by hot HPH in which a liquid solution containing molten lipids and dissolved drug (tretinoin) is incorporated into a hot aqueous solution containing surfactants and chitosan under high agitation using a homogenizer to form a pre-emulsion [8]. The pre-emulsion was then homogenized by HPH (three cycles at 600 bar) and cooled in an ice bath to form the NCs.

Nanoprecipitation, also known as solvent displacement or interfacial deposition of preformed polymers, consists of the addition of an organic solution (lipids, surfactants and polyesters dissolved in an organic solvent) containing retinoids into an aqueous solution containing surfactants. The organic solvents are then eliminated and the aqueous phase is concentrated by evaporation under reduced pressure (Figure 4) [12,98–99]. Interestingly, variations such as the use of lipids derived from retinoids (retynil palmitate) to form the core of NCs [100,103] and chemically modified amphiphilic cyclodextrins to form the coating of retinoid-loaded NCs [101] have been proposed.

Szczepanowicz *et al.* proposed a simple method consisting of the addition of an organic solution (retinol and anionic surfactant oils solubilized in organic solvents) into a cationic polymer solution, enabling the adsorption of the polymer onto the surfactant [104]. After formation of the polymeric layer, consecu-

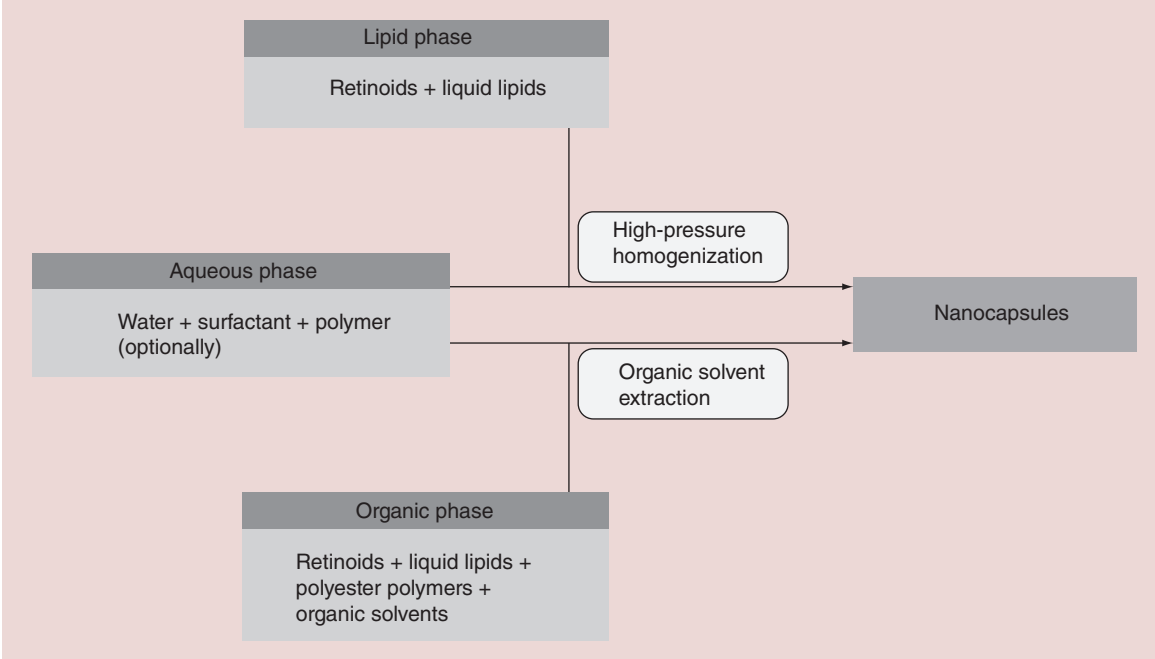

Figure 4. Preparation of nanocapsules by hot high-pressure homogenization and nanoprecipitation by solvent extraction (interfacial deposition of preformed polymers).

tive layers of polyelectrolytes were formed using the layer-by-layer technique.

NCs developed by the methods depicted above have a size range between 100 and 285 nm and low polydispersity indices (0.02–0.376) [8,12,98–99,101–103]. Coatings composed of polyesters polymers (poly-ε-caprolactone and polylactic acid), chemically modified polymers (with PEG) and lipid–cyclodextrins have shown ζ-potential values ranging between -3 and -29 mV [98–100,105]. Chitosan-coated NCs have shown highly positive ζ-potentials (~60 mV) [8]. In general, all systems show very high EE (≥90%), with the exception of those comprising lipid–cyclodextrins (17–30%; Table 1) [101].

Retinoid-loaded lipid nanoparticles incorporated in semisolid vehicles: formulation & fabrication

Although most of the published work in lipid nanoparticles reviewed above focuses on the nanoparticle itself; said systems are frequently incorporated into secondary vehicles, such as hydrogels or creams, for topical delivery [109]. While the majority of the work conducted to date involves the incorporation of SLNs or NLCs into secondary vehicles, we expect further research describing studies of NEs or NCs incorporated into said systems in order to build on the few works reported to date [88,92,99]. Nevertheless, we could anticipate that the fluid nature of NEs and NCs may have an impact on the capacity of these systems to sustain drug release in comparison with what has been described for SLNs or NLCs.

In general, formulations can be obtained by: incorporating the lipid nanoparticles into pre-existing products; viscosing agents can be added to the continuous phase of the manufacturing process of the nanoparticles in order to yield a hydrogel or gel-like formulation; or by direct production in the lipid nanoparticle manufacturing process (Figure 5) [29]. In this last case, the fabrication of the final topical formulation consists of a single-step process.

For the production of lipid nanosystem-enriched hydrogels, various gelling agents can be used, such as linear (cellulose derivatives including hydroxyethyl cellulose and hydroxypropyl methylcellulose) or branched polysaccharides (xanthan gum, Carbopol polymers and chitosan, among others; Table 1). Adequate physical stability has been reported for systems containing SLNs and NLCs [64,65,108,109].

In two early reports on the incorporation of vitamin A-loaded SLNs into secondary vehicles, Jenning *et al.* proposed the use of a cream and a hydrogel [64,65]. For the gel, the process consisted of the preparation of a 0.5% xanthan gum gel followed by the addition of the SLN aqueous dispersion under stirring. The cream was prepared by heating the two phases separately, mixing the lipid and aqueous phase and then cooling to 40°C, after which the SLN dispersion was added. In another study, Carbopol 940 (1%), Pemulen™ TR-1 (1%; Lubrizol, OH, USA), xanthan gum (1%), and Lutrol® F 127 (15%) gels (BASF) were manufactured and compared as secondary vehicles [4]. Apart from the gel-forming polymer, glycerol (10%) and an aqueous vitamin A-loaded SLN dispersion (20%) were added to the formulation.

The fabrication of final products containing lipid nanoparticles in one step usually consists of using high lipid concentrations. As the concentration of lipid increases, the viscosity of the final formulation increases to the point of an almost solid product [110,111]. Regardless of the high lipid content and the high viscosity of the product, it has been reported that particle sizes can be kept below 300 nm [111].

In vitro characterization of lipid nanoparticles & secondary vehicles enriched with lipid nanoparticles containing retinoids

The characterization of topical vehicles enriched with lipid nanoparticles can either focus on the combination of the secondary vehicle with the nanosystem or on the nanosystem itself. The following sections delve into the most common methods employed to characterize lipid nanoparticle-enriched secondary vehicles.

Particle size

Dynamic light scattering and laser diffraction have been previously used as means to characterize size distributions in systems enriched with lipid nanoparticles [112–117]. In one example using laser diffraction, Pardeike *et al.* have indicated that from size distribution profiles of creams as secondary vehicles, one can expect to detect a peak of particle sizes in the same range as the nanoparticles, as well as a peak describing the micron-sized oil droplets of the cream used as the secondary vehicle [112]. For the characterization of nanoparticles incorporated into hydrogels, dynamic light scattering can be similarly used without the influence of micron-sized particles or vesicles. Jenning *et al.* demonstrated that different hydrogels had different effects over average particle sizes and distributions of vitamin A-loaded SLNs [65]. For example, weak polar (glucuronate) or uncharged polysaccharides (xanthan gum and hydroxyethyl cellulose) had little effect on average size and were correlated with a slight increases in the polydispersity index from 0.25 to 0.3. On the other hand, carrageenan and hydroxypropyl distarch phosphate, which are polysaccharides with highly polar groups, exhibited a marked increase in particle size

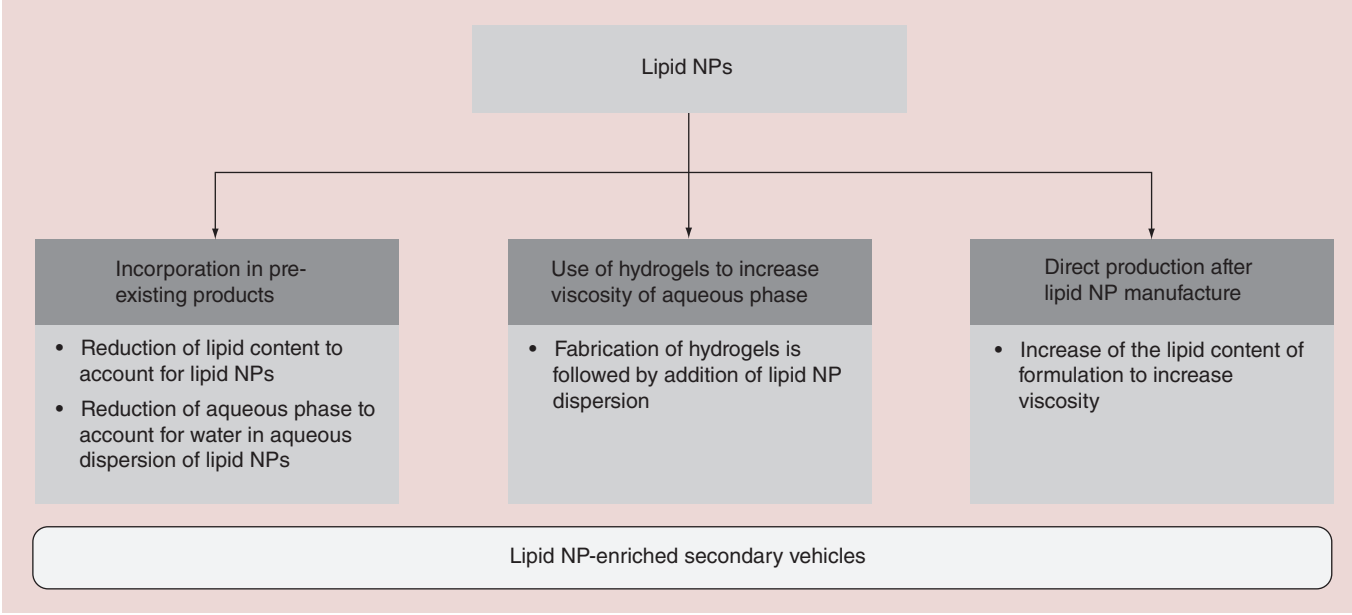

Figure 5. Methods of fabrication of lipid nanoparticle-enriched secondary vehicles. Notable differences between the systems are indicated by bullet points.
NP: Nanoparticle.

and polydispersity indices, indicating the induction of aggregation [65]. In another study of lipid nanoparticle-enriched hydrogels, Müller *et al.* observed that the use of sodium hydroxide to neutralize a polyacrylic acid gel was associated with an increase in particle aggregation [118]. This has been previously attributed to the neutralizing effect that sodium ions have, decreasing the ζ-potential and thus leading to aggregation.

Drug release

In general, for lipid nanoparticles, drug release is partly dependent on where the drug is located within the therapeutic system. In a capsule-like morphology, if the active is located in the outer shell, then burst release could be observed with little controlled release. On the other hand, in a matrix-like morphology, if the active is homogeneously distributed throughout the matrix, then controlled release can be expected. The physical structure of the lipid nanoparticle could also determine the drug release profile; for example, solid matrices (SLNs and NLCs) could modify drug release compared with more fluid nanocarriers containing liquid cores (NEs and NCs).

Different strategies for determining drug release have been employed in the literature [4,65]. Release kinetics have been described to not only depend on the delivery system itself, but also on the experimental conditions (e.g., sink or nonsink conditions and the release medium, among others). Furthermore, due to the nanoparticulate nature of these systems, release experiments can be conditioned by the method of sample separation (filtration, centrifugation or dialysis) [36]. Jenning *et al.* have reported two methods for studying the influence of different variables on the release of vitamin A from SLNs [65]. A first method involves the use of Franz diffusion cells and isopropyl myristate-soaked cellulose nitrate filters (0.1-µm pore diameter) as membrane models in order to simulate the stratum corneum lipophilic properties. Due to the characteristics of the method, water evaporation was allowed and thus a concentration gradient increase could occur, leading to increases in flux over time. Another reported setup consisted of a test tube filled with the aqueous SLN dispersion and covered with Miglyol in order to serve as receiving compartment. In these experiments, an increase in release rate was evidenced in SLNs dispersed in buffer in comparison to NEs containing vitamin A, which was attributed to an induction of transformation into the more stable form of the lipid and thus faster expulsion of vitamin A from the SLN.

Permeation studies

A permeation study (also referred to as penetration) is perhaps the most important *in vitro* characterization study that a topical formulation can be subjected to. It serves as an approximation to the *in vivo* setup and has been described thoroughly for lipid nanoparticle systems loaded with retinoids [4,7,13,15,64,67–68,106]. It is important to note that differences in the skin model, type of diffusion cell, temperature, receiving media, dose application and diffusion area can all significantly

Table 1. Examples of solid lipid nanoparticles, nanostructured lipid carriers, nanoemulsions and nanocapsules containing retinoids for topical administration developed during the past 10 years.

Characteristic	SLNs	NLCs	NEs	NCs
Selected retinoids	Isotretinoin [7,15,67,106], ATRA [62–63,74], tretinoin [13,68], tretinoin [14]	Acitretin [60], isotretinoin [7,26], ATRA [69], tretinoin [14]	Tretinoin [12,88–89], vitamin A palmitate [90], adapalene [91], isotretinoin [99]; retinyl palmitate [92]	Isotretinoin [99], tretinoin [8,12,105], retinyl palmitate [100,103], vitamin A palmitate [107]
Drug association efficiency/ theoretical drug loading (%)	80,6 [106], >95 [62,74], 35–50 [13], 93.5 [63], 80–100 [67], 38–45 [68], 86.25 [14], 89.5 [7,15]	63 [60], 78.6 [26], 92.1 [14], >95 [69]	99 [99], >99.9 [12]/11.1 w/w in the oil phase [90], 1 w/w [92], 0,1 w/v [91], 0.3 w/w [83]	99 [99], >99.9 [12], >99 [8], >95 [105], 17–30 [101]
Secondary vehicle	Xanthan gum [13,108], Carbopol® Ultrez 10 (Lubrizol, OH, USA) [13,68], Carbopol 940 [13,15,108], Carbopol ETD 2020 [13]	Carbopol 934 [14,26,60]	Carbopol 940 [92], Carbopol Ultrez 20 [92], Pemulen™ TR-1 NF [92], hydroxyethyl cellulose [92], gel (composition not reported) [88]	Hydroxyethyl cellulose and imidazolinidyl urea [99], Carbopol [100,103,105], hydroxyethyl cellulose [101,107]
In vitro efficacy/ biological evaluation	Accumulation in skin (mouse and human skin) and avoidance of systemic uptake [106]; less irritating (Draize test on rabbits) than commercial gel [106]; less irritating than marketed cream (mouse skin) [74]; gel containing SLNs is less irritating than marketed cream [13]; reduced drug photodegradation and *in vitro* permeation (rat skin) comparable to marketed cream [13]; lower irritation (Draize test on rabbits), greater tolerance and slower drug release in SLN-based gels than the commercial product [68]; enhanced photostability, skin transport and antipsoriatic activity than vesicular carriers and the marketed formulation [14]	NLC gel shows higher deposition in human skin than plain gel [60]; NLC gel improves clinical response and reduces local side effects [60]; increased effect against *Propionibacterium acnes* [7]; accumulation in the epidermis and dermis (rat skin) [7]; enhanced efficacy on photoaged skin (mouse) compared with marketed products [26]; better tolerated (Laca mouse skin) than marketed products [26]; enhanced photostability, skin transport and antipsoriatic activity than vesicular carriers and the marketed formulation [14]	Enhanced drug retention on mouse skin [88]; NE penetrates through hair follicles in porcine skin [91]	Twofold higher photostability than methanol solution [12]; noncytotoxic to HaCaT cells and high antibacterial effect against *P. acnes* and *Staphylococcus aureus* [8]; cytotoxicity and phototoxicity in HaCaT and 3T3 cells only at high concentrations [103]; hydrogels containing NCs show a third of the photodegradation compared with the formulation with nonencapsulated drug [105]
General comments	Prolonged drug release due to solid core, highly selectable size ranges and available equipment for scaling up	Higher drug association and stricter control over drug release due to increased drug solubility in liquid lipids in comparison with SLNs, highly selectable size ranges and available equipment for scaling up	High drug association, high physical fluidity and available equipment for scaling up	High drug association, potentially active surface, high physical fluidity and less safe than the others systems

ATRA: All-*trans* retinoic acid; NC: Nanocapsule; NE: Nanoemulsion; NLC: Nanostructured lipid carrier; SLN: Solid lipid nanoparticle.

impact on the data, making it difficult to compare results between studies. Due to the restrictions associated with primate research, the next most relevant skin model from animals is the porcine skin [119]. However, due to its availability, it is the skin of rodents (mice, rats and guinea pigs) the most used *ex vivo* model in permeation studies [119]. The existence of hairless species (nude mice and hairless rats), make these models easier to handle experimentally and mimic the human skin better than hairy skin [120].

One strategy for the sample collection and description of active molecule distributions is to assay the donor, receptor and membrane (skin model) for the amount of drug after a permeation experiment in a diffusion cell. In one study on human cadaver skin mounted on Keshary–Chien diffusion cells, Pople and Singh quantified vitamin A concentrations in the receptor compartment, the donor compartment and the skin by extraction in suitable solvents at the end of the permeation study [4]. A marked difference between gels enriched with a SLN-encapsulated vitamin A formulation and a nonencapsulated conventional gel was evidenced with the use of this methodology. Vitamin A-loaded SLNs accumulated to a greater extent in the skin and very little permeated through into the receptor compartment of the cell, in contrast to the nonencapsulated vitamin A form. This effect was attributed to the nanosize of this colloidal carrier allowing its permeation into the skin and, due to its lipidic nature, it could concentrate and accumulate in this tissue [4].

A different strategy for investigating permeation through the skin of vitamin A derivatives in lipid nanoparticles consists of sampling from the receiving chamber in a diffusion cell at predetermined time points in order to illustrate the permeation kinetics and then determine the flux of the active. The permeation rate (also known as the flux or permeation coefficient) can be obtained from the slope of the linear portion of the cumulative amount permeated per unit area versus time plot. For example, Liu *et al.* investigated the permeation capacity of isotretinoin-loaded SLNs dispersed in an aqueous medium on rat skin mounted on vertical diffusion cells [67]. At the end of the 8-h permeation study, no presence of isotretinoin could be detected in the receiving compartment, in comparison with a 0.06% isotretinoin tincture from which the active permeated at a flux of 0.76 μg/cm^2/h. Conversely, it was found that isotretinoin accumulated in the skin in all SLN formulations regardless of particle size [67]. In another study with pig skin as the model in Franz flow-through diffusion cells, vitamin A-loaded SLNs incorporated either into a cream or a hydrogel were found to permeate to deeper regions of the skin in comparison with free vitamin A-loaded secondary vehicles [64]. It was also described that loaded SLNs in the hydrogel permeated to deeper regions of the skin in comparison with SLNs in a cream due to the difference in solubility of vitamin A in the secondary vehicle.

Occlusivity & transepidermal water loss measurements

As drug carriers for topical delivery, lipid nanoparticles have been described to have occlusive properties due to their film formation capacity in skin, reducing the transepidermal water loss [121]. In transepidermal water loss experiments, a slight decrease in water loss has been described due to the use of SLNs dispersion [64]. A more marked effect of localization in the upper layers of the skin was evidenced when an occlusion-sensitive active such as retinyl palmitate was encapsulated in SLNs and these were incorporated in either a hydrogel or a cream [64]. Similar studies were conducted in gels enriched with tretinoin-loaded SLNs [68]. Regardless of the gel formulation, a marked decrease in water loss could be found in comparison with the negative control and a marketed cream due to the film formation associated with the use of SLNs.

Efficacy

As mentioned above, retinoids have an antimicrobial effect against the bacteria involved in acne. This feature has also been identified previously in retinoid-loaded lipid nanoparticles [7,8]. Ridolfi *et al.* have reported on the fabrication of chitosan–tretinoin-loaded SLNs and have evaluated their antimicrobial efficacy on standard stocks of *Propionibacterium acnes* and *Staphylococcus aureus* [8]. It was found that the incorporation of tretinoin in chitosan-loaded SLNs increased the antibacterial activity in comparison with tretinoin-loaded SLNs. This was attributed to the inherent antimicrobial activity of chitosan described in the literature [122,123]. More recently, Raza *et al.* reported on *in vitro* the antimicrobial activity of isotretinoin-loaded lipid nanocarriers such as NLCs and SLNs against *P. acnes* [7]. In their investigations, the authors found that isotretinoin has an inherent antimicrobial effect against *P. acnes*; however, by incorporating the active into lipid nanocarriers, a twofold enhancement of activity was found, as well as an increase in drug permeation through mice skin. The enhancement of antimicrobial activity was attributed to the interaction between the lipids in the nanocarriers and the bacteria cell wall, which results in an increased time of interaction and the sustained delivery of isotretinoin [7]. Importantly, in this context, the authors have demonstrated that isotretinoin and adapalene

included in SLNs and NEs were able to improve the effect of the drugs in animal models of acne [15] and also in human patients [16,17]. Interestingly, Raza *et al.* [14] and Agrawal *et al.* [60] demonstrated that tretinoin and acitretin loaded in hydrogels enriched with SLNs and NLCs, respectively, offered enhanced antipsoriatic activity when compared with appropriate controls (liposomes, ethosomes and hydrogels) and a marketed product (Table 1).

In vivo evaluation methods of lipid nanoparticles loaded with retinoids

Skin biopsy

Histological examination, although invasive, is of relevance for the study of drug disposition and drug effect through skin layers. Jeon *et al.* have reported on an *in vivo* antiwrinkle study of a retinyl palmitate-loaded SLN system in female hairless mice (HR-1) [124]. Histological inspection of elastic fiber expression under the microscope revealed that all retinoid treatments showed preventative effects against the degradation of elastic fibers by ultraviolet irradiation. In a different investigation, Pople and Singh have reported on skin hydration *in vivo* studies using topical gels enriched with SLNs of vitamin A or conventional gels in albino rats [4]. After 24 h of treatment, the animals were sacrificed and the skin was inspected microscopically and the thickness of the stratum corneum was measured. The studies revealed an increase in the thickness of the stratum corneum with improved skin hydration after treatment as compared with conventional vitamin A gels.

Tape stripping

In tape stripping experiments, adhesive films are successively applied to and removed from the skin after the administration and penetration of the topically applied substances [125–127]. With each tape strip, the corneocytes are removed layer by layer, and subsequently, the amount of the applied substances that penetrated to the removed layer is determined. Shiva *et al.* have compared the skin permeation of isotretinoin-loaded SLNs with a commercial isotretinoin gel in humans [106]. The isotretinoin gel formulation exhibited higher concentrations of the active in more superficial layers, while the same layers contained lower amounts of isotretinoin with the SLN treatment. The overall results obtained in this study showed that SLNs can offer improved topical delivery of isotretinoin compared with the commercial gel in terms of better skin tolerability and higher and deeper skin accumulation [106]. In a similar study, hydrogels incorporating either isotretinoin-loaded poly(ε-caprolactone) NCs or free isotretinoin were compared in terms of cutaneous penetration. Using the tape stripping method in excised human and pig skin, an increase in skin penetration of NCs in the stratum corneum of both skin models was found [99]. Furthermore, the penetration of adapalene-loaded NEs for transfollicular delivery has been investigated by tape stripping of porcine ear skin. The results showed that adapalene penetration in the hair follicles was associated with the increase in water content of NEs [91].

Draize test

For topical formulations, the Draize test [128] is used to evaluate and compare the skin irritation potential of formulations, which is one of the major disadvantages associated with tretinoin therapy (erythema), strongly limiting its utility and acceptability by patients. Shah *et al.* used a rabbit model to evaluate the irritation potential of a SLN-enriched tretinoin gel in comparison to a marketed tretinoin cream [13]. The results indicated that the SLN-enriched tretinoin gel caused remarkably fewer erythematic episodes compared with the marketed tretinoin cream (Figure 6), clearly indicating its potential for improving the skin tolerability of tretinoin. In this context, it has been demonstrated in rabbits that the use of natural lipids in SLNs has been associated with positive results in a skin irritancy test [68]. In addition, Shiva *et al.* have evaluated the irritation potential of isotretinoin-loaded SLNs in comparison with a commercial isotretinoin gel in rabbits [106]. After 72 h of application, isotretinoin-loaded SLNs caused considerably less irritation, demonstrating the potential of SLNs to improve the skin tolerability and patient acceptance of isotretinoin.

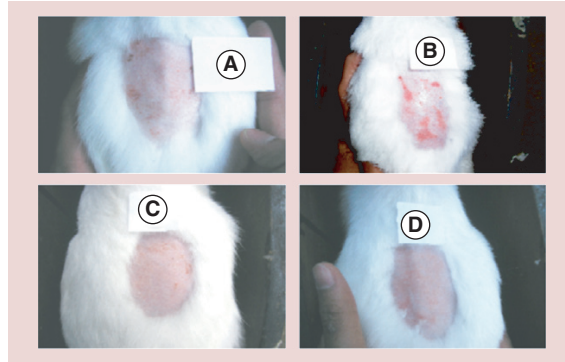

Figure 6. Skin irritation studies on New Zealand rabbits. Skin irritation studies on New Zealand rabbits of **(A)** a control group, **(B)** the Retino-A® (Janssen-Cilag, India) marketed cream formulation, **(C)** solid lipid nanoparticle gels without tretinoin and **(D)** treitinoin-loaded solid lipid nanoparticles in gel. Photographs were taken 24 h after application of the formulation.
Reproduced with permission from Elsevier [13].

The major results both *in vivo* and *in vitro* of selected formulations of SLNs, NLCs, NEs and NCs from the last 10 years are summarized in Table 1.

Conclusion & future perspective

The tremendous potential of retinoids for cosmetic and therapeutics purposes has promoted an exhaustive study of traditional/new formulations for delivering these actives. This, together with the demonstrated possibility of lipid nanoparticles to significantly improve the targeting of active drugs into the skin, represents a great motivation to deliver retinoids in lipid nanocarriers. The chance of suspending SLNs, NLCs, NEs and NCs in secondary vehicles (aqueous media, gels and creams) allows for the obtaining of final formulations with high acceptance by customers/patients. To date, several of these retinoid-containing formulations have been demonstrated to improve the effects of commercial products for the treatment of acne and psoriasis. The use of safe excipients in formulations (usually used in cosmetics), together with the large challenge of preventing skin aging, will drive the rapid development of new cosmetic nanoformulations for treating wrinkles and facial pigmentary disorders. These developments will represent new challenges to industries in terms of the scale up of formulations. As SLNs and NLCs have been investigated for years now, it could be expected that new products enabled by these technologies will become available in the near future. NEs and NCs, although heavily investigated for several delivery routes, could still be distant from market. This is particularly relevant for NCs, in which the polymer coating could impose further safety concerns. Considering the different characteristics of each skin disorder (e.g., acne, psoriasis and aging, among others) treated with retinoids and the physicochemical differences between retinoids, the development of an ideal and unique formulation is complicated. Thus, the lipid nanoparticle, drug and secondary vehicle should be adequately selected in order to specifically treat the target tissue associated with the specific disorder.

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Executive summary

Background

- The capacity of lipid nanoparticles to efficiently encapsulate and protect retinoids, in addition to their potential for being incorporated into secondary semisolid vehicles, provides evidence that they may be important delivery systems for skin treatment.
- Retinoids are lipophilic drugs with demonstrated skin efficacy for increasing elasticity, decreasing roughness, reducing keratoses and controlling acne and psoriasis.

Lipid nanoparticles loaded with retinoids

- Retinoids loaded in solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), nanoemulsions (NEs) and nanocapsules (NCs) can be fabricated with technology that is available to industry. Methods can be classified in terms of the energy applied during the process.
- In general, excipients that are normally used in creams with therapeutic/cosmetic purposes are used to elaborate SLNs, NLCs, NEs and NCs.

Retinoid-loaded nanoparticles incorporated into semisolid vehicles

- Formulations can be obtained by: incorporating nanoparticles into pre-existing products; adding viscosing agents to the continuous phase of the manufacturing process; or by direct production in the lipid nanoparticle manufacturing process.
- Among the available *in vitro* study approaches for characterizing these complex formulations, we highlight particle size, drug release, membrane permeation, occlusivity and transepidermal water loss and efficacy. Among the available *in vivo* study approaches for characterizing these complex formulations, we mention skin biopsies, tape stripping and Draize test.

Conclusion & future perspective

- Semisolid vehicles containing retinoids loaded in SLNs, NLCs, NEs and NCs represent an important approach for the skin treatment of aging, keratoses, acne and psoriasis.
- Approaches to the technological and scientific challenges should focus mainly on the optimum permeation/retention of retinoids at target sites and nanoparticle production on a large industrial scale.

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