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Nanostructured lipid carriers: Promising drug delivery systems for future clinics

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Abstract

During the past decade, the number of studies describing *nanostructured lipid carriers* (NLCs)-based formulations has been dramatically increased. The raise in NLC exploitation is essentially due to defeated barriers within the technological process of lipid-based nanoparticles' formulation and increased knowledge of the underlying mechanisms of transport of NLCs via different routes of administration. This review article aims to give an overview on the current state of the art of NLC as controlled drug delivery systems for future clinics through novel NLC applications providing examples of successfull outcomes. The reported data clearly illustrate the promise of these nanoparticles for novel treatments in the near future.

From the Clinical Editor: The understanding of the nanostructured lipid carriers (NLC)-based formulations has improved with continuing research recently. The result has seen an increase in the use of these in the clinical setting. In this comprehensive review, the authors discussed the current state and major challenges in the use of nanostructured lipid carriers as controlled drug delivery systems.
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Key words: Nanostructured lipid carriers; NLC; SLN; Lipid nanoparticles

In the early 90s, Professor R.H. Müller (Germany) and Professor M. Gasco (Italy) started to investigate the potential of a new nanoparticle-based formulation: the so-called *solid lipid nanoparticles* (SLNs).[1,2] Their formulation was based on lipids and presented, among others, the advantage of avoiding an organic solvent during the preparation method, contrary to existing organic nanoparticles (e.g. PLGA nanoparticles), and presenting a high stability in vivo as they remained solid at body temperature. The latter characteristic made them an alternative not only to organic nanoparticles but also to previous lipid-based formulations (e.g. liposomes). However, an important drawback appeared to compromise the future applicability of the formulation: the low drug loading. Further investigations on the formulation helped on the improvement of the SLNs. The incorporation of a liquid lipid to the solid matrix of the nanoparticle was found to increase the number of imperfections in the core solid matrix, thus facilitating the incorporation of an increased amount of drug, while preserving the physical stability of the nanocarriers. This new unstructured-matrix SLN was designed as *nanostructured lipid carriers* (NLCs) (Figure 1).[3-5]

From the onset of the description of NLCs as newly developed drug delivery systems to the present, the number of publications based on NLC formulations has considerably increased. There are two main reasons that can explain this success: (i) the beaten hurdles in lipid-based nanoparticles development (e.g. imaging, stability) and (ii) newly emerged applications for NLC formulations (e.g. colitis, P-gp efflux inhibition, theranostics).

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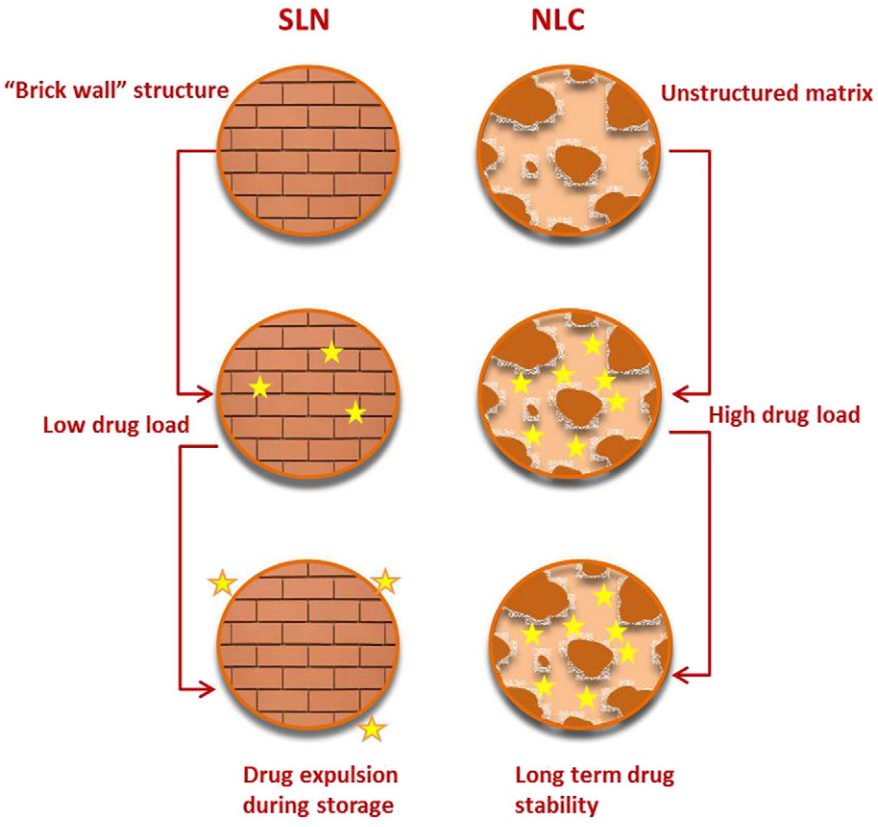

Figure 1. Schematic illustration of SLN structure versus NLC structure, where NLC advantages over SLNs are highlighted.

The present review is focused on NLC formulations and aims (i) to illustrate the major challenges to be addressed in drug delivery when using lipid nanoparticles (LPs), while revealing the principal achievements on defeating these barriers, and (ii) to give an extensive overview on the newly emerged applications of NLC formulations as drug delivery systems by different administration routes.

NLC formulation

NLC composition

NLCs consist of an unstructured solid lipid matrix made of a mixture of blended solid and liquid lipids and an aqueous phase containing a surfactant or a mixture of surfactants. Typically, solid lipids are mixed with liquid lipids in a ratio of 70:30 up to a ratio of 99.9:0.1, whereas the surfactant content ranges 1.5%-5% (w/v).[6] Throughout literature, different combinations of lipids and/or surfactants have been described and most of these are included in Table 1. All the components described below are commercially available in marketed products and/or approved by different regulatory agencies (Generally Recognized As Safe (GRAS), USA FDA IIG).[7,8]

NLC preparation

Although there are several methods adopted in order to prepare NLCs (e.g. microemulsification, solvent displacement), the high pressure homogenization process is preferred to the rest of the methods as no solvents are needed within the preparation. The hot surfactant solution is added to the blended and melted lipids containing the drug (~10 °C above the melting temperature). The resulting microemulsion is subsequently homogenized under high pressure obtaining a hot nanoemulsion. Usually, a pre-emulsification step is performed prior to the homogenization process. The nanoemulsion is then cooled and the NLCs formed.[9,10]

The high pressure homogenization process can be easily translated from a lab scale production (batch size 2 l) to a large scale production (batch size >50 l) in the pharmaceutical industry while avoiding the use of any organic solvent within the preparation procedure.[2]

Altogether, the use of GRAS components, the large-scalable production methods for their preparation and the improved drug safety demonstrated by the use of lipid-based nanocarriers make NLCs an ideal drug delivery system candidate for the pharmaceutical market.[11]

Overcoming physicochemical barriers

The major obstacle hampering lipid-based nanoparticle formulation has been its innate lipid nature that hindered the physicochemical characterization of the nanoparticles by routing techniques.[12] LPs can be visualized using sophisticated techniques (e.g. transmission electron microscopy (TEM)), which are aggressive and compromise nanoparticle structure

Table 1
Lipid and surfactant excipients used for NLC formulation and examples of marketed products containing these excipients.

Component	Chemical name	Trade name examples	Examples of marketed products
Solid lipid	Glyceryl palmitostearate	Precitol ATO®5	Xifaxan® (Salix Pharmaceuticals Inc.)
	Glyceryl dibehenate	Compritol®888 ATO	–
	Cetyl palmitate	Crodamol™ CP	Azelex® (Allergan)
	Stearic acid	–	Viokace™ (Aptalis Pharma Inc.)
	Tripalmitin	–	Survanta® (Abbot)
Liquid lipid	Caprylic/Capric triglycerides (C_8/C_{10})	Miglyol®812 and 810, Labrafac™, Softison 378	Avodart™ (Glaxo SmithKline)
	Vitamin E and derivatives (TOS, TPGS)	–	Neoral® (Novartis)
	Lauroyl Polyoxylglycerides	Gelucire®44/14	Lipofen® (Cipher Pharmaceuticals, Inc.)
	Monoacylglycerols	Myverol 18-99 K	Terramycin® (Pfizer)
	Soy lecithin	Epikuron™200	Baycip® (Bayer)
	Squalene	–	Cutanova (Dr. Rimpler Gmb)
Surfactants	Polysorbates	Tween®20, Tween®80	Targretin® (Ligand)
	Poloxamers (188, 407)	Lutrol®F68, Lutrol®F127	Rapamune® (Wyeth-Ayerst)
	Macrogol-15-hydroxystearate	Solutol®HS 15, Kolliphor® HS 15	Oxidize® (Beta S.A.)
	Polyoxiethylene stearate	Myrj 52	Dermazene™ (Stratus Pharmaceuticals Inc.)
	Polyoxyl castor oil	Cremophor EL	Kaletra® (Abbot)

integrity. The captured images are poorly depicting LP appearance. Another important issue is their stability during storage. These nanoparticles are only possibly preserved with the aid of a cryoprotectant and/or preservative, already compromising the initial physicochemical properties (size and surface charge, mainly) of LPs. Finally, the in vivo visualization of lipid nanoparticles has been questioned as the lipophilic dyes used for tracking LPs (e.g. Nile Red) are claimed to be released from the nanoparticles labeling the surrounding tissues where nanoparticles are assumed to be accumulated, haltering the track of the nanoparticles themselves. Fortunately, these critical issues have recently undergone through a promising evolution.

Stability of NLCs

Among the hurdles in LP development, the long-term physical instability of LP dispersions is undoubtedly a major concern. Even if NLCs present reduced water content in comparison with SLNs, any product containing water should be preserved from bacteria growth and preserve the initial nanoparticle properties (e.g. particle size). There are mainly two possibilities available toward nanoparticle dispersion preservation: (i) to remove the water content from the nanoparticle suspension and convert the suspension into a solid (freeze-drying)[13] or (ii) to maintain the water present in the dispersion and add a so-called *preservative*.[14]

Ideally, a freeze-dried nanoparticle should preserve the stability of nanoparticles while maintaining unaltered the initial nanoparticle parameters (e.g. nanoparticle size). Hence, it should have an adequate appearance, present a short reconstitution time, as long as being easily resuspended in water, and induce no changes on particle size distribution of nanoparticles, while maintaining the encapsulated drug activity.[15] However, as a general rule, lyophilization of LPs implies aggregation in the absence of a cryoprotectant.[9] Beloqui et al evaluated trehalose, sucrose and sorbitol at different concentrations (5, 10 and 15% (w/v)) as cryoprotectants for NLC formulations.[16-19] The freeze-dried samples were resuspended and then analyzed for appearance, rehydration rate and by measuring the mean particle size and zeta potential. Only trehalose resulted effective for preventing/avoiding NLC aggregation at all assayed concentrations (5, 10 and 15% (w/v). Interestingly, trehalose was also described to favor the stability of the structure of the shell in other LPs, lipid nanocapsules.[20,21] Varshosaz et al studied different carbohydrate and polymeric cryoprotectants as candidates for NLC lyophilization.[13] The authors evaluated Microcelac® (mixture of lactose and Avicel), Avicel PH102 (microcystalline cellulose), mannitol, sucrose and Avicel RC591 (mixture of microcrystalline cellulose and sodium carboxymethyl cellulose) at different concentrations, and concluded that Avicel RC591 at 1% concentration exhibited the lowest increase on NLC particle size. In spite of the promising results regarding NLC lyophilization, it should be taken into account that the use of a cryoprotectant often implies the alteration of nanoparticle surface initial properties and, thus, an alternative preservation method might be advisable.

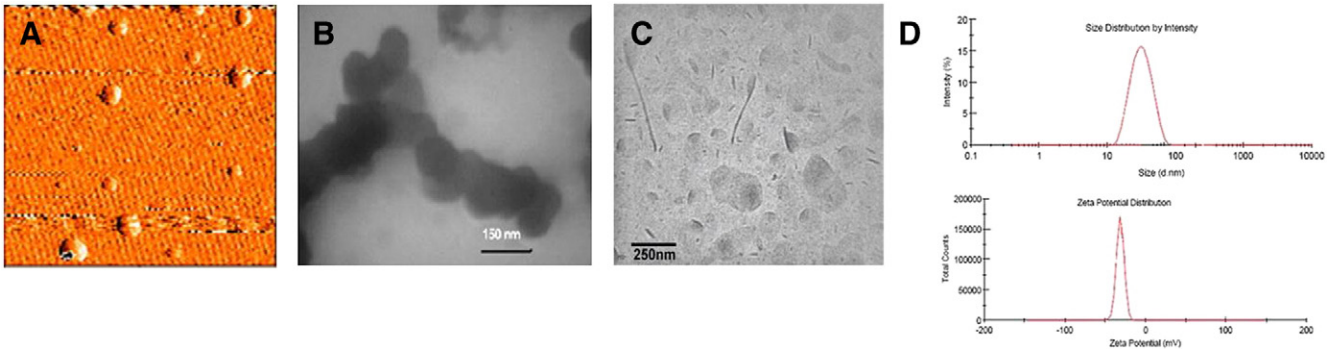

Figure 2. AFM **(A)** and TEM **(B)** images of valproic acid loaded NLCs. A Cryo-TEM image of unloaded NLCs is presented in **(C)**. Size and zeta potential distribution of mangiferin-loaded NLCs measured by DLS **(D)**. Images reprinted and adapted with permission from Elsevier and John Wiley and Sons.[13,28,29]

NLCs can possibly be preserved dispersed in water.[14] This can be possibly achieved by the addition of a preservative agent. Obeidat et al studied the influence of 11 different preservatives on the size, zeta potential and physical stability of a Q10-loaded NLC dispersion after 3, 6 and 12 months of storage at room temperature.[14] Interestingly, seven out of the eleven studied preservatives were identified as suitable for the preservation of NLC dispersions, with Hydrolite 5 being the best choice to stabilize Q10-loaded NLCs. The use of preservatives toward NLC storage might be beneficial when compared to the freeze-drying method. As a general rule, the ideal preservative for NLCs should be as hydrophilic as possible, non-ionic (in order to minimize changes in zeta potential) and with little affinity to the particle surface.[14]

NLC morphology

For the characterization of nano-scale drug delivery systems, dynamic light scattering (DLS), electron microscopic methods and atomic force microscopy (AFM) are commonly employed in order to obtain reliable information about the morphology and structural properties of nanocarriers, and have been employed also to investigate NLCs.[22-25]

The DLS technique provides information about the volumetric mean diameter of an amount of particles. In the case of NLCs, the factors affecting the preservation of the structural integrity are both the same for Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM). Shrinkage of the nano-systems can occur due to dehydration of the samples and drying can cause structural changes which usually end up in electron microscopic images that do not correspond to the original formulation morphology.[23] Moreover, conventional electron microscopy is predisposed to artefacts when using surfactant solutions, hampering the interpretation of the obtained images. Techniques based on cryofixation can help to overcome these major drawbacks. The cryo electron microscopy has been developed in order to evaluate the nanocarrier native structure in a frozen-hydrated estate and to preserve the hydrated nano-specimen initial morphology, and has been successfully employed for the investigation of the structural properties of NLCs.[26,27] In addition, it is possible to obtain information about the internal structure of the nanocarriers (e.g. oil droplets present inside NLCs). The AFM technique does not require a prior sample preparation and it is possible to measure nanoparticles in suspension avoiding artefacts formation or nanoparticle morphology alteration.[24] Figure 2 illustrates different NLC-based formulations' images obtained by AFM, TEM, Cryo-TEM and size and zeta potential distribution measured by DLS.

Fluorescent dye labeling of LPs

An important issue when investigating the biodistribution or cellular trafficking of nanocarriers by fluorescence dye labeling, is the stability of the labeling when this is not grafted into the lipid-based nanocarriers. Bastiat et al formulated the key question on this regard: *"How can it be validated that the fluorescence observed during an experiment corresponds to the nanocarriers, and not to the free dye released from the nanocarriers?"*.[30] To find solution for the posed question, the authors developed an innovative method to study the labeling stability when a lipophilic fluorescent dye is encapsulated inside a lipophilic nanocarrier. The method is based on the ability of the fluorescent dye to be transferred from its hosting nanocarrier to a lipophilic compartment. The authors concluded that, following a partition property dependent on oil volume modifications, dyes like Nile Red (NR) or Coumarin-6 (Cou-6) could be transferred from the lipid nanocarriers to an oily medium and could enter blank lipid nanocarriers from a lipophilic compartment. However, they demonstrated that the fluorescent labeling of lipid nanocarriers using the indocarbocyanine dye family (DiI, DiO, DiD) is irreversible and non dye diffusion occurs from the lipid nanocarrier. Thus, this labeling ensures the nanocarrier localization within the cell. The difference on dye diffusion from lipid nanocarriers could be explained by the localization of NR and Cou-6 in the core of lipid nanocarriers, thus being transferred to external lipophilic compartments, whereas DiI, DiO or DiD is trapped in the shell of the nanocarriers, not being transferred.

This new method to determine dye diffusion from lipid nanocarriers represents an alternative to determine the efficient fluorescent dye encapsulation and further labeling stability of lipid nanoparticles. Moreover, it highlights the potential of the indocarbocyanine dye family for efficient fluorescent dye labeling of lipid nanocarriers.

NLCs: arising applications by different routes of administration

After presenting the major hurdles to overcome regarding lipid based nanoparticle formulation and characterization, we

herein provide a review on the major achievements on NLCs through different routes of administration (e.g. dermal, oral, pulmonary, ocular) highlighting the ultimate applications of this drug delivery system, thus helping fulfill its potential.

Dermal route

The stratum corneum (SC) is the main barrier to the percutaneous absorption of topically applied drugs. It is a multilayer matrix of hydrophobic and hydrophilic components whose structural integrity is maintained by the presence of modified desmosomes, called corneodesmosomes, which lock the corneocytes together and provide tensile strength for the SC to resist to shearing forces.[31] The barrier nature of the SC depends critically on its unique constituents, such as the ceramides that account for 45%-50% of the hydrophobic lipids present in the intercellular spaces, cholesterol (25%), long-chain free fatty acids mostly with chain lengths C22 and C24 (15%), and 5% other lipids (e.g. cholesterol sulfate, cholesterol esters, and glucosylceramides).[32] These lipids, which are organized in multilamellar bilayers, regulate the passive flux of water through the SC and are considered to be very important for skin barrier function.[33]

The absorption of drugs through the skin can occur through intact epidermis (transepidermal route) and/or skin appendages (transappendageal route).[34] Since skin appendages occupy <0.1% of the total human skin surface, the transappendageal route has generally been considered to contribute minimally to the overall permeation. However, recent advances in this area have demonstrated the important role of hair follicles as penetration pathways and reservoir structures for topically applied compounds.[33] This is particularly important when topically applied drugs are formulated in nanoparticulate systems because it has been demonstrated that the penetration depth of the particles can be influenced by their size resulting in the possibility of a differentiated targeting of specific follicular structures.[35] Nevertheless, under normal circumstances, the predominant route is the transepidermal, with a diffusion path length larger than the thickness of the SC (20 μm) and that has been estimated to be as large as 500 μm. Importantly, the intercellular spaces contain structured lipids and a diffusing molecule has to cross a variety of lipophilic and hydrophilic domains before it reaches the junction between the SC and the viable epidermis.[33]

To overcome low absorption rates, lipid-based nanoparticulate systems and vesicular colloidal carriers have been investigated not only to enhance percutaneous absorption but also for drug targeting to the skin or even to its substructure. Thus, they might have the potential for an improved benefit/risk ratio of topical drug therapy.[6] Actually, topical application, either for therapeutic or cosmetic purposes is a research area in which NLCs have been used since the 1990's with promising results.[36] The literature is rich on publications on the ability of lipid-based particles and vesicular colloidal carriers to penetrate the SC and the possibility of using such particles for topical drug delivery has been widely discussed. In addition, the cosmetic field was the first to benefit from the positive features of LPs, having marketed several products, including Nanobase® (Yamanouchi) and a series of Q10-containing NLC-based anti-aging treatment products from Cutanova (Dr. Rimpler GmbH).[6,37]

LPs protect incorporated active molecules against chemical degradation and allow modulating drug release, thus leading to the use of labile active ingredients, which cannot be utilized in traditional formulations.[38] In addition, they are reported to enhance the bioavailability of topically applied drugs, increasing skin penetration. Their small particle size ensures close contact to the SC and thus, the amount of encapsulated drug reaching the site of action will be increased. To explain this phenomenon, Müller et al[36] proposed a model of film formation on the skin dependent on the particle size, thus causing an occlusion effect after application of SLNs and NLCs onto the skin. Therefore, topical application of aqueous SLN or NLC dispersions creates a mono-layered lipid film onto the skin, which prevents transepidermal water loss, thus increasing skin's moisture and hydration.[39] Due to their rigidity and the surfactants in their composition, the thermodynamic stability of SLNs and NLCs is enhanced. The presence of surfactants also plays a role on skin's permeability because they may cause skin's structure disruption. This mechanism has been pointed out as allowing a physical UV blockage, thus explaining the putative sunscreen effect of LPs[40] and attempts have also been made to explore the potential of NLCs in sunscreen-loaded formulations with promising results in terms of drug loading and stability.[41]

Undoubtedly, LPs present occlusive properties, which facilitate drug permeation through SC. However, the drug penetration may also be affected by parameters such as the carriers themselves, the type and concentration of the lipid, and the drug localization in the nanoparticle structure.[42,43] Research on the mechanism of interaction between NLCs and the skin showed a clear change in intercellular packing after topical application of an NLC dispersion, with reduced corneocyte packing and wider inter-corneocytes gaps. It was hypothesized that NLCs had an effect on the skin barrier, thus promoting drug permeation.[43] Studies performed with NR-labeled NLCs showed not only that the nanoparticles promoted distribution and penetration into the skin ex vivo, with epidermal targeting, but also that they may constitute a suitable reservoir system for transdermal delivery with a good in vivo/in vitro correlation.[44,45]

Suitable formulation in NLCs may therefore control drug penetration in the skin. Studies on the cutaneous absorption of betametasone valerate into excised human skin showed the importance of a close association of drug molecule and carrier, while emphasizing the role of drug localization within the lipid matrix.[42] The latter may be influenced by drug physicochemical properties, surfactant type and concentration, lipid type, and production method. Therefore, besides the good tolerability, simple and cost-effective large-scale production, stability, targeting, controlled drug release, and protection of liable drugs from degradation it is very important that the drug is soluble in the lipid matrix. In NLCs this is enabled by the oil (liquid lipid) that solubilizes the drugs to a much higher extent than solid lipids.[38]

Concerning the transappendageal route, it is well known that sebaceous glands are of particular interest for topical delivery of corticoids for treating diseases like seborrheic dermatitis, although scientific publications demonstrating skin permeation

through follicular orifices are scarce.[33,46] However, follicular penetration has also been studied for NLCs, since hair follicles represent interesting target sites for topically applied substances, such as diphencyprone, minoxidil[47,48] and spironolacone[49] for topical treatment of alopecia. Once they penetrate into a hair follicle, particles can follow different routes, according to their size thus providing some sort of selective targeting. Smaller particles can penetrate through the follicular epithelium into blood circulation.[35]

Many studies have demonstrated the great potential of NLCs, either for local drug delivery or to improve drug absorption by the skin, with a wide variety of drug molecules intended for topical treatment of multiple diseases, making the dermal route perhaps the most studied for the application of NLCs (Table 2). It should be noticed that the number of reports in the literature involving SLNs is much larger.

Importantly, NLCs must be either applied as an aqueous dispersion or incorporated in suitable liquid or semi-solid preparations for cutaneous use that provide an appropriate formulation consistency for application upon the skin. The development of such pharmaceutical dosage forms containing SLNs or NLCs usually involves a) the incorporation of SLNs/NLCs in preformed topical products (e.g. lotions, gels or creams); b) addition of viscosity enhancers to the aqueous phase of SLNs/NLCs to obtain a gel (e.g. xanthan gum, hydroxyethylcellulose, hydroxypropyl methylcellulose, Carbopol® and chitosan); or c) the direct production of a final product containing only nanoparticles in a one-step process using high lipid concentrations.[6] Attention should be paid to avoid and excessive dilution of the lipid nanoparticles and therefore of the encapsulated drug, as well as instability phenomena such as particle aggregation or dissolution.

The literature clearly indicates that skin penetration depends not only on nanoparticle formulation, but also on the vehicle in which NLCs are included. Vitorino et al[77,78] reported a Carbopol® hydrogel as a vehicle for NLCs, combined with classical skin enhancers (ethanol and limonene). The combination of the occlusive effect promoted by NLCs, with the SC lipid disturbance attributed to the chemical enhancers, arises as an appealing strategy to overcome stratum corneum, resulting in transdermal delivery.[45,77] These authors successfully co-encapsulated two lipophilic drugs – simvastatin and olanzapine – in tripalmitin-based NLCs with properties adequate for sustained drug transdermal delivery over 48 h. The in vitro skin experiments showed that the external medium in the NLC dispersion strongly influenced permeation. Moreover, it was also seen that the use of NLCs determined a synergistic effect with the selected permeation enhancers, ethanol and limonene, thus promoting marked flux enhancement ratios, relative to the drugs in solution. Converting the formulation into a gel reduced the flow rate. A correlation between enhancer positioning in the lipid bilayer and enhancement effect was suggested from molecular dynamics studies that corroborated the experimental results.[77] In vivo pharmacokinetic studies confirmed systemic absorption of the co-encapsulated drugs reaching therapeutic plasma concentrations. Furthermore, the previous use of microneedles did not significantly enhance drug permeation, supporting the use of passive methods as suitable for a transdermal delivery system and suggesting the NLC formulation as a promising transdermal delivery system for both co-encapsulated drugs.[45] Transdermal absorption of other therapeutically relevant drugs encapsulated in NLCs has also been described (Table 3).

Oral route

While increasing highly lipophilic drugs' bioavailability, NLCs exhibit a prolonged residence time in the GIT when compared to other lipid-based formulations, and present a different release mechanism that can be modulated by tunning the lipids contained within their solid lipid matrix. In general, the digestion speed and extent of lipid microemulsions (e.g. Self Emulsifying Drug Delivery systems (SEDDS)) are higher than those for NLCs.[86] Due to these special features, and according to the experimental data described within the present section, NLCs represent a promising delivery system for lipophilic drugs that present a low bioavailability and are especially indicated for gastrointestinal diseases like inflammatory bowel diseases (IBDs).

Increased muco-penetration

After oral administration, at the instestinal site and prior to reaching the underlying epithelium, the nanocarriers must confront the mucus layer, a relevant barrier regarding nanoparticle penetration. Charged nanoparticles can interact electrostatically with the mucus and get retained within the mucus not reaching the cell surface. Moreover, the mucus mesh-pore size conditions nanoparticle penetration. Altogether, the development of mucus-penetrating nanoparticles remains challenging.[87]

Beloqui et al[88] investigated the effect of the intestinal mucus layer on SQV-loaded NLC uptake. For this purpose, they compared SQV permeability across Caco-2 (no mucus) and Caco-2/HT29-MTX (mucus model) cell monolayers using different NLC formulations.[10] With all the formulations assayed, SQV permeability values were significantly lower across Caco-2/HT29-MTX cell monolayers when compared to Caco-2 cell monolayers, indicating that the mucus layer hinders NLC access to the underlying epithelium and represents a barrier to overcome for NLC formulations.

In addition, these authors demonstrated that the coating of NLCs with a dextran–protamine complex (Dex–Prot) significantly increased (up to ~2-fold) SQV permeability in the Caco-2/HT29-MTX cell model. The higher permeability was related to the nanometer-size of NLCs along with the surface charge, close to neutrality, which helped in diminishing the electrostatic interaction with the mucus, avoiding the mucus entrapment and, therefore, increasing NLC penetration across the mucus layer.

Mechanism of transport of NLCs across the intestinal barrier and P-gp drug efflux inhibition

Improved knowledge of the mechanisms and processes involved in nanoparticle transport across the intestinal barrier and the physicochemical properties limiting their transport can help in designing more efficient nanocarriers to enhance oral drug absorption.[10] Beloqui et al evaluated the potential of NLCs to enhance the oral bioavailability of saquinavir (SQV), a BCS class IV drug and P-gp substrate, and studied NLC transport

Table 2
Dermal applications of NLC formulations.

Encapsulated drug	NLC Composition	Outcome	Ref.
Acitretin	Precirol ATO 5/oleic acid/Tween 80	Clinical studies with psoriasis patients showed significant improvement in therapeutic response and reduction in local side effects with NLCs.	50
All-trans retinoic acids	Oleic acid/Cetyl palmitate/Cineole/Limonene/ Transcutol/Butylated hydroxytoluene/ Tween 20/Tween 80	NLCs showed higher epidermal permeation across the skin, suggesting their potential use as dermal drug delivery carriers for all-trans retinoic acids.	51
Artemether	Gelucire 43/01/Transcutol/Phospholipon 85G	Artemether permeates excised human epidermis, where the formulation served as a reservoir to gradually control drug release over an extended period of time.	52
Benzocaine	Compritol 888 ATO/Miglyol 812/Lutrol F68	Radiant heat tail-flick test was carried out in mice to determine the antinociceptive effect of benzocaine from NLCs. The results showed NLCs act as an effective drug reservoir, prolonging the anaesthetic effect.	53
Celecoxib	Compritol 888 ATO/Miglyol/ 1,2-dioleoyl-sn-glycero-3- [(N-(5-amino-1-carboxypentyl) imidodiacetic acid) succinyl nickel sal/ Tween 80. NLCs modified with cell penetrating peptides	Cell penetrating peptides increased the permeability of celecoxib encapsulated in NLCs. In vivo pre-treatment with the formulation inhibited PGE2 and IL-6 expression compared to controls.	54
	Glyceryl dilaurate/Capmul MCM/ Cremophor RH 40/Transcutol	The NLC-based gel showed faster onset and elicited prolonged activity until 24 h.	55
Clotrimazole	Glyceryl tripalmitate/Miglyol 812/Tyloxapol	Only in vitro characterization was performed. NLCs presented prolonged release properties.	56
Coenzyme Q10	Cetyl palmitate/Mygliol 812/Tego-Care 450	Only in vitro characterization was performed.	57
	Cetyl palmitate/Medium chain triglycerides/ Polyglyceryl-3 methylglucose distearate	Q10-loaded NLCs showed in vitro a 10-fold increased accumulation in epidermis when compared to a Q10-containing emulsion	58
	Cetyl palmitate/Cetiol OE 4.00/ Span 20/Tween® 80	Ultra-small NLCs (80 nm) improved dermal delivery of Q10 when compared to 230 nm NLCs.	59
Cyproterone	Precirol ATO 5/Miglyol 812/Poloxamer 188	Drug encapsulation into NLCs resulted in a 2- to 3-fold increase in absorption.	60
Diphenciprone	Precirol/Squalene/Pluronic F68/Lecithin	Follicular uptake by NLCs was 2-fold higher for DPCP compared to the free control. Great accumulation of NLCs in the follicles and the deeper skin strata.	48
Econazole	Precirol ATO 5/Oleic acid/Poloxamer 407	Drug loaded NLCs showed better permeability and thermodynamic stability as effective topical delivery system for deep-seated fungal infection.	61
Epidermal growth factor (rhEGF)	Precirol ATO 5/Miglyol 812/ Poloxamer F68/Tween 80	The bioactivity of the NLC formulations was even higher than that of free rhEGF. Topical administration of NLC-rhEGF improved wound healing in terms of wound closure, restoration of the inflammatory process, and re-epithelisation grade.	62,63
Finasteride	Precirol ATO 5/Mygliol 812/Tween 60	Loading efficiency was 70%-90% and formulations showed low penetration levels in pig ear skin.	64
Fluconazole	Compritol 888 ATO/oleic acid/ Pluronic F68/lecithin	In vivo skin-retention studies showed drug with a 5-fold higher accumulation in the case of NLC formulation, which also revealed maximum antifungal efficacy.	65
Flufenamic acid	Precirol ATO 5/Mygliol 812/Plantacare 810	Good in vitro skin permeation and penetration properties for flufenamic acid formulated in NLCs.	66
Fluticasone propionate	Precirol ATO 5/Labrasol/Tween 80/lecithin Precirol ATO 5/Softigen 767/Tween 80/lecithin	Reduction of adverse effects/not demonstrated	67
Indometacin	Compritol 888 ATO/Miglyol 812/Lutrol F68	The anti-inflammatory effect, following topical application, was more prolonged with the NLC gel formulation. Topical bioavailability in the SC depended from the formulations.	68
Ketoconazole	Compritol 888 ATO/α-tocopherol/ Poloxamer 188/sodium deoxycholate	Only in vitro characterization was performed. In contrast to SLNs, the NLCs were able to	69

(continued on next page)

Table 2 (*continued*)

Encapsulated drug	NLC Composition	Outcome	Ref.
		stabilize the drug.	
Lidocaine	Compritol/Miglyol/Lutrol F68	Results from the radiant heat tail-flick test showed NLCs can act as an effective drug reservoir, prolonging the anaesthetic effect.	53
Loratadine	1-Hexadecanol/Mygliol 812/TegoCare 450	Penetration profiles of drug encapsulated in NLCs were lower when compared to a nanoemulsion	70
Lutein	Glyceryl tripalmitate/Mygliol 812/ Plantacare 810 Carnauba wax/Mygliol 812/Plantacare 810	Permeation studies with fresh pig ear skin showed little NLC-formulated or free lutein, thus remaining in the skin and not being systemically absorbed.	71
Metotrexate	Witepsol S51/Oleic acid/ Tween 60 or Tween 80	NLCs promote drug skin penetration in vitro.	72
Minoxidil	Stearic acid/Oleic acid/Poloxamer 188	Only physicochemical characterization was performed.	73
	Precirol ATO 5/Squalene/ Pluronic F68/Lecithin	NLCs reduced minoxidil penetration through the skin. This may indicate a minimized absorption into systemic circulation. Great accumulation of NLCs in the follicles and the deeper skin strata.	48
	Tristearin/Oleic acid/Cholesterol/ Lecithin/Tween 80	Only physicochemical characterization was performed. In vitro release suggests a faster onset and prolonged activity up to 16 h.	47
	Cetyl palmitate/Oleic acid/Tween 60	Loading efficiency was <30% and formulations showed low penetration levels in pig ear skin.	64
Oxybenzone	Glyceryl monostearate/Mygliol 812/ polyvinyl alcohol Glyceryl monostearate/Oleic acid/ polyvinyl alcohol	The incorporation of oxybenzone into NLCs greatly increased the in vitro sun protection factor and erythemal UVA protection factor of oxybenzone more, while providing the advantage of overcoming side effects of free oxybenzone.	74
Quercetin	Glyceryl monostearate/Stearic acid/ Medium chain triglycerides/ d-α-Tocopheryl polyethylene glycol 1000 succinate	NLCs promoted drug permeation, increased drug retention in epidermis and dermis, and enhanced the anti-oxidant and anti-inflammatory effects.	75
Spironolactone	Compritol 888 ATO/Olive oil/ Transcutol/Tween 80	Confocal laser scanning microscopy confirmed the potential of delivering NLCs within the hair follicles, suggesting the possibility of localized delivery into the scalp hair follicles.	49
Valdecoxib	Glyceryl dilaurate/Capmul MCM/ Cremophor RH 40/Transcutol	The NLC-based gel showed faster onset and elicited prolonged activity until 24 h.	76

mechanisms across the intestinal barrier.[10,89] Three NLC formulations differing in particle size (165, 247 and 1000 nm, formulation A, B and C, respectively) and surfactant content were obtained, all negatively charged. All three formulations significantly increased SQV permeability across Caco-2 cell monolayers when compared to SQV in suspension, although formulation B did it to a higher extent compared to formulation A and C (3.5-fold and 2-fold increment).

The mechanistic study of SQV loaded NLCs across the intestinal barrier revealed differences in the mechanisms of transport of the NLC formulations across the intestinal barrier. When SQV was encapsulated in formulation B (particle size 247 nm), both clathrin and caveolae were involved in SQV transcytosis across Caco-2 cell monolayers, whereas only clathrin was involved in SQV transcytosis of formulation A and C. These differences in the mechanisms of transport could explain the differences observed on SQV permeability values.

In a second step, to evaluate whether the NLCs inhibited the P-gp drug efflux, SQV permeability studies were conducted in Caco-2 cells under verapamil inhibition, a well-known P-gp inhibitor. Formulations A and C exhibited greater permeability when the P-gp efflux was inhibited, whereas no difference in the permeability rates with formulation B were observed regardless of the presence or absence of verapamil, suggesting that this formulation circumvented the P-gp efflux and, thus, enhanced SQV permeability. The involvement of both mechanisms of internalization, clathrin and caveolae, observed for formulation B could explain the ability of formulation B to circumvent the P-gp drug efflux. Figure 3 represents the different mechanisms of transport observed for the three NLC formulations.

These findings highlight the importance of the composition of NLCs designed for oral drug delivery, as well as their physicochemical properties (size and surface properties), toward oral bioavailability enhancement, and the ability of NLCs to circumvent the P-gp drug efflux.

Oral bioavailability enhancement

One of the most widely exploited routes of administration for NLCs exploitation has been the oral route.[3,4,90,91] Due to the increased drug loading capacity of NLCs, most of these studies have been focused on the ability of NLCs to improve the oral bioavailability of poorly water-soluble drugs (classified Biopharmaceutics Classification System (BSC) II or IV). Table 4 describes some examples of highly lipophilic drugs whose oral bioavailability increases when formulated as NLCs.

Table 3
Transdermal applications of NLC formulations.

Encapsulated drug	NLC Composition	Outcome	Ref.
Buprenorphine	Precirol 5 ATO/Squalene/Myverol/ Lecithin/Pluronic F68	Buprenorphine encapsulated in NLCs produced high drug/prodrug permeation. The skin permeation of buprenorphine could be adjusted within a wide range by combining a prodrug strategy and lipid nanoparticles.	79
Flurbiprofen	Dynasan 114/Lecithin/Caprylic/ capric triglycerides/Tween 80	The pharmacokinetics of flurbiprofen in rats following application of SLN gel and NLC gel for 24 h were evaluated. The C_{max} and AUC of the NLC formulation were 1.8 and 2.5 times higher than those of the SLN gel formulation, respectively. Drug bioavailability with reference to oral administration increased 4.4-fold when gel formulations were applied. Anti-inflammatory effect was higher for NLCs and SLN formulations than the orally administered drug.	80
	Stearic acid/Soybean oil or Olive oil or Castor oil/Lecithin/ Tween 20	Bioavailability of flurbiprofen from NLCs was over 1.7-fold that of the commercial gel. The NLCs showed a quick onset and sustained anti-inflammatory effect over 24 h.	81
Ketorolac	Compritol 888 ATO/Mygliol 812/ Lutrol F68	Ketorolac prodrugs were compared to ketorolac-containing NLCs. Skin permeation of prodrugs was significantly enhanced, compared with ketorolac. NLCs were ineffective in increasing ketorolac percutaneous absorption. NLCs seemed more appropriate for sustained release owing to the possible formation of a drug reservoir into the skin.	82
Lornoxicam	Compritol 888 ATO/Lanette/ Oleic acid/Pluronic F68	NLCs showed the lowest rate of drug penetration through rat skin, compared to nanoemulsions and SLNs. NLCs increased drug penetration through rat skin compared to a drug-containing gel.	83
Meloxicam	Cetyl palmitate/Caprylic acid/ Tween 80/Propylene glycol	NLCs demonstrated sustained release and enhanced the skin permeation and deposition of meloxicam especially into the dermis.	84
Nitrendipine	Dynasan 114/Lecithin/Caprylic/ capric triglycerides/Tween 80	The antihypertensive activity in vivo was compared to that of oral nitrendipine. NLC gel controlled hypertension from the first hour.	80
Sildenafil	Cetyl palmitate/Glycerol monolinoleate/ Span 85/Propylene glycol	Transdermal permeation across human skin was assessed using a modified Franz diffusion cells. NLCs significantly enhanced in vitro release and transdermal permeation.	85
Simvastatin + Olanzapine	Tripalmitin/Oleic acid/Tween 80	Olanzapine and simvastatin were coencapsulated in NLCs. Chemical penetration enhancers, limonene and ethanol, added to the NLC formulations, promoted a synergistic permeation enhancement of both drugs. Pharmacokinetic parameters showed a transdermal effect, with NLCs presenting better in vivo performance than the control gel. In vivo experiments in rats correlated well with in vitro findings.	45,77

Inflammatory bowel disease treatment

Inflammatory bowel diseases (IBDs) are chronic and relapsing inflammatory disorders in which the physiological properties of the intestinal barrier are altered. A disrupted intestinal barrier, increased mucus production, and, hence, a thicker mucus layer, and the infiltration of immune-related cells are some of the hurdles encountered by the nanocarriers. Due to the physical characteristics of the inflamed intestinal tissues, a selective accumulation of the nanocarriers at the intestinal site targeting the inflamed region is desirable.[106-108]

LPs represent a promise as drug delivery systems in the treatment of inflammatory disorders due to their ability to modulate the immune system.[109] Furthermore, lipid based therapy has been described as a potential therapeutic strategy in the treatment of IBDs due to the lack of physiological lipids described in patients suffering this pathology.[110] Beloqui et al[107] evaluated the potential of budesonide-loaded NLCs (BDS-NLCs) in reducing inflammation in colitis. First, they evaluated in vitro the anti-inflammatory potential of BDS-NLCs in reducing TNF-α secretion from lipopolysaccharide (LPS)-activated macrophages. Macrophages were pre-incubated for 4 h with BDS in suspension, unloaded NLCs or BDS-NLCs and subsequently exposed to LPS activation. After 24 h, both loaded and unloaded NLC formulations significantly reduced tumor necrosis factor (TNF)-α secretion when compared to untreated LPS-activated macrophages. In vivo, the authors evaluated the anti-inflammatory potential of BDS-NLCs in a murine dextran-sulfate (DSS)-induced colitis model. After a 3-day treatment, only BDS-NLCs significantly reduced the neutrophil infiltration and TNF-α and interleukin (IL)-1β expression in the colon when compared with the non-treated-DSS group, while maintaining the mucosal architecture similar to the healthy group. However, no significant differences were observed from the healthy group when mice were treated with either

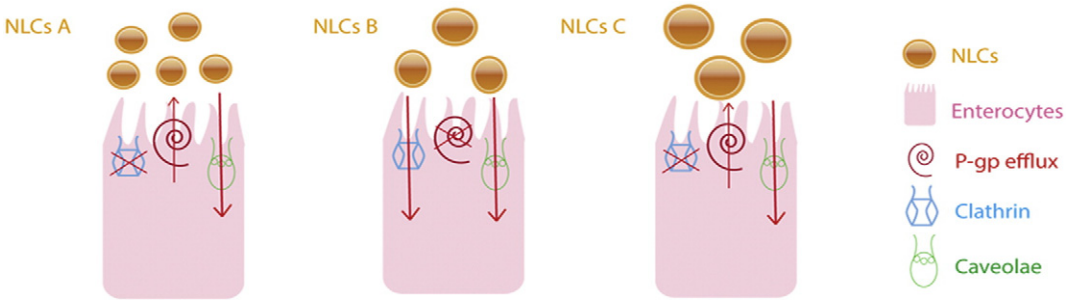

Figure 3. Illustration of SQV loaded NLC transport mechanisms across the intestinal barrier. Image reprinted with permission from Elsevier.[10]

Table 4
Highly lipophilic drugs encapsulated within NLCs for oral bioavailability enhancement.

Encapsulated Drug	BCS class	NLC size (nm)	Outcome	Ref.
Etoposide	IV	<130	Increased etoposide oral bioavailability up to 3.5-fold in comparison to etoposide in suspension.	92
Fenofibrate	II	<200	Higher C_{max} levels and higher AUC (4-fold increase) in comparison to fenofibrate in suspension.	93
Iloperidone	II	~160	Increased oral bioavailability up to 8.3-fold in comparison to iloperidone in suspension.	94
Lovastatin	II	<300	$AUC_{0-\infty}$ value of lovastatin after orally administered was up to 6.4-fold higher for NLCs than lovastatin in suspension.	95
Luteolin	II	~45	Improved oral bioavailability of luteolin.	86
Miconazole	II	~200	Equal therapeutic effect of a commercial oral gel formulation using a 17-fold lower dose.	96
Montelukast	II	<200	A ~143-fold oral bioavailability enhancement after a single dose compared to drug suspension.	97
Quercetin	II	~30	Increased quercetin aqueous solubility ~1000-fold.	98
Saquinavir mesylate	IV	~250	Saquinavir transport across Caco-2 cells was enhanced up to 3.5-fold by NLCs compared to drug suspension.	10,89
Resveratrol	II	<200	Higher physical stability and negligible resveratrol release when compared to SLNs.	99
Silymarin	III	<100	A ~2.5- and ~3-fold higher bioavailability was achieved with silymarin loaded NLCs when compared to marketed Legalon® capsules and silymarin pellets, respectively.	100
Simvastatin	II	<300	Superior pharmacotechnical properties than SLNs and drug suspension: higher bioavailability, delayed T_{max} and prolonged $t_{1/2}$.	101
Spironolactone	II	~150	Shift in the metabolic profile of spironolactone when compared to a spironolactone syrup.	16
Tamoxifen	II	~200	Tamoxifen loaded NLCs enhanced the bioavailability by 2.7-fold and prolonged the $t_{1/2}$ by 7.10-fold when compared to tamoxifen in suspension.	102,103
Tripterine	III	<150	$AUC_{(0-t)}$ of tripterine loaded NLCs was 4.8-fold higher than tripterine suspension.	104
Vinpocetine	II	<200	The relative bioavailability of vinpocetine loaded NLCs was 322% compared to vinpocetine suspension.	105

unloaded-NLCs or BDS in suspension. NLCs were detected in the colon of DSS-treated mice 12 h after oral administration. These results justify the use of NLCs for anti-inflammatory drug delivery in the gastrointestinal tract (GIT) and give venue to further investigations on the anti-inflammatory potential of lipid based nanocarriers in IBD treatment.

Pulmonary delivery

In the past years, the improvement of inhaled drug delivery systems has fostered the progress in the treatment of local lung diseases, such as lung cancer, cystic fibrosis, bronchiectasis, respiratory infections or chronic obstructive disease.[111,112] Drug delivery to the lungs is challenged by rapid clearance, and a local and prolonged drug release is desirable when targeting this organ.[113] Nanotechnology has been a vastly exploited strategy toward local pulmonary delivery and the variety of nanoparticles studied is large (e.g. polymeric nanoparticles, micelles, liposomes, solid lipid nanoparticles). LPs have specially gained attention due to the increased drug loading for poorly soluble drugs and a presumably better acceptability of lipidic excipients, although it should be taken into account that to date few are regulatory-approved excipients for respiratory drug delivery.[114] Particularly, solid lipid-based nanocarriers (SLNs and NLCs) have been widely studied for pulmonary application.[115]

Cipolla et al[114] have published a review on pulmonary drug delivery based on the preclinical development and clinical studies in humans regarding lipid-based carriers. In the authors' opinion, several gaps need to be filled prior to translating solid lipid-based nanoparticles into the clinics, such as (i) increased knowledge of the mechanisms of transport of solid lipid-based nanoparticles in the lungs, (ii) the physicochemical characteristics of solid lipid-based nanoparticles (e.g. drug loading, physical stability) need to be improved in order to fulfill pulmonary formulation requirements and (iii) further in vivo studies evaluating the effect of repeated administrations and (iv) their immunogenicity are needed. Nevertheless, important improvements have been made in NLC formulations addressing the aforementioned issues.

A severe limitation for transport of nanomedicines within the lungs is their limited translocation across the respiratory

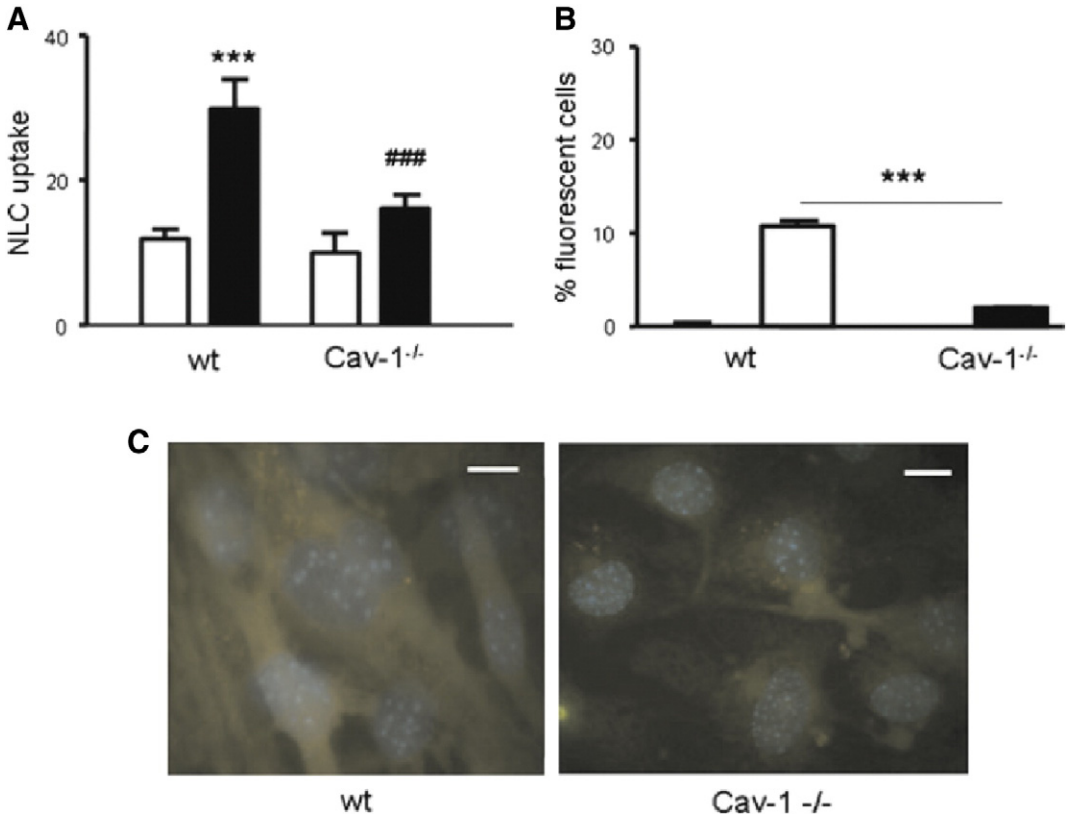

Figure 4. Uptake of curcumin-loaded NLCs in wt and Cav-1$^{-/-}$ LMVECS, highlighting caveolae role on NLC transport across these endothelial cells, is represented quantitatively **(A** and **B)** and qualitatively **(C)**. **(A)** Fluorescence levels of untreated (control) cells (white bars) and cells incubated with curcumin-loaded NLCs at a 3.3 mg/mL concentration for one hour (black bars). **(B)** Percentage of fluorescent cells relative to all cells measured by flow cytometry. **(C)** Images under fluoresecent microscope (×1000 augmentation). Reproduced with permission from Springer.[119]

epithelia toward the systemic circulation.[116] However, translocation of radiolabeled SLNs into the lymphatics has been described.[117,118]

Increased knowledge on the mechanisms of transport of the nanocarriers across the respiratory epithelia could help develop more effective nanomedicines. Kardara et al[119] evaluated the mechanisms of transport of curcumin-loaded NLCs across human and mouse lung microvascular endothelial cells (LMVECs) isolated from wild-type (wt) and Caveolin-1 knockout (Cav-1$^{-/-}$) mice. The authors concluded that in cells lacking caveolin-1, the uptake of NLCs was diminished and, thus, NLCs need caveolar vesicles for their internalization. These results are consistent with previous studies evaluating NLC mechanism of transport across different cell lines.[10] The intracellular uptake of curcumin-loaded NLCs in wt and Cav-1$^{-/-}$ LMVECS is presented in Figure 4. The authors further investigated the potential of NLCs on protecting from endothelial barrier leakiness, an initial step in alveolar edema formation. For this purpose, Immortilized Human Pulmonary Microvascular Endothelial cells (HPMEC-ST1) monolayers were challenged with thrombin to induce endothelial permeability. Co-incubation with NLCs preserved this effect in a dose-dependent manner and prevented actin cytoskeletal reorganization and intercellular gap formation. These observations were assessed in vivo in an acid aspiration murine acute lung injury model (ALI). Mice were pre-treated with an intravenous injection of NLCs or saline prior to 0.1 N HCl intratracheal administrations. The results concluded that NLCs preserve lung microstructure in ALI, showing no microstructural lesions in mice receiving NLCs.

These results provide new insights into the mechanisms of transport of NLCs across the pulmonary endothelium and raise hope for pulmonary inflammatory diseases' treatment.

An increasing number of NLC formulations for pulmonary purposes have been published toward different pulmonary treatments. The ability of NLCs to reach the lungs, their pharmacokinetics and their efficacy have been evaluated in different in vivo models (e.g. lung cancer or lung inflammatory diseases). Table 5 summarizes the physicochemical properties of NLCs studied through the pulmonary route and the in vivo models where they have been tested.

Altogether, (i) the absence of in vitro toxicity, (ii) the demonstrated lung targeting efficacy, (iii) the increased knowledge of the mechanisms of transport of NLCs across the respiratory epithelia and (iv) the in vivo efficacy in different lung disease models justify the application of NLCs to treat pulmonary diseases.

Lung cancer

Lung cancer is the leading cause of cancer death worldwide reaching 1.59 million deaths in 2012 according to the WHO (http://www.who.int/mediacentre/factsheets/fs297/en/). Chemotherapy represents the primary lung cancer treatment. However,

Table 5
NLC formulations for pulmonary administration.

Drug	Size (nm)	Zeta potential (mV)	Polydispersity index	Encapsulation Efficiency (EE) (%)	Drug Loading (%)	Outcome	Ref.
Celecoxib	217 ± 20	−25.30	0.200	>90	4	In vivo lung deposition and pharmacokinetic studies in male Balb/c mice	120
Dexamethasone	~200	2.3 ± 1.2	–	88.2	2.1	In vivo macrophage targeting ability anti-inflammatory potential in a carrageenan air pouch inflammation rat model	121
Doxorubicin	110 ± 20	+45-+65	0.400	–	5	In vivo antitumor efficacy in an orthotopic model of lung cancer	122
	~200	+19	0.130	86.70	–	In vivo antitumor efficacy against A594 solid tumors in mice	123
Itraconazole	~100	−33.3 ± 0.4	0.273	98.78	0.4	–	124
Montelukast	~185	36.3 ± 1.8	0.239	~95	0.2	Improved bioavailability, longer residence time and targeting factor of 11.76 for montelukast compared to drug solution in Wistar rats	97
Paclitaxel	110 ± 20	+45-+65	0.400	–	5	In vivo antitumor activity in an orthotopic model of lung cancer	122
Sodium colistemethate	252.5 ± 20.3	−26.1 ± 7.05	0.339	94.79	–	In vivo biodistribution studies demonstrated increased NLC deposition in B6SJLF1 mice lungs and antimicrobial activity against *Pseudomonas aeruginosa*.	125

chemotherapeutics are related to multidrug resistance of cancer cells and systemic side effects as a result of nonspecific anticancer drug localization. Local pulmonary drug delivery is desirable over (i) oral drug delivery, due to poor oral bioavailability of anticancer drugs, or (ii) intravenous drug delivery, due to high doses toxicity-related side effects on healthy organs.[122] Nucleid acids have been identified as efficient suppresors of proteins responsible for drug resistance in different cancer cell lines, including lung cancer cells.[126-128] However, they are not suitable for intravenous administration due to their poor pharmacokinetics. Combining the synergistic effect of chemotherapeutics and nucleic acids treatment mechanisms appears to be a promising strategy in order to overcome chemotherapy-related tumor resistance via pulmonary route.[122,123,127,129-131]

NLCs have been highlighted as efficient drug delivery carriers for anticancer drugs[132] and have been now described to efficiently co-deliver both chemotherapeutics and nucleic acids.[122,123] Taratula et al[122] described a multifunctional NLC-based system containing (i) an anticancer drug (doxorubicin (DOX) or paclitaxel (TAX)), (ii) siRNA targeted to *MRP1* mRNA as a drug resistance suppressor, (iii) siRNA targeted to *BCL2* mRNA and (iv) an analog of luteinizing hormone-releasing hormone (LHRH) (a ligand for lung cancer cells' membrane receptors). In vitro, LHRH-DOX-NLCs complexed with FAM-labeled siRNA (emiting green fluorescence) successfully delivered both DOX and siRNA into human A549 lung cancer cells. Moreover, LHRH-TAX-NLC-siRNA significantly decreased the expression of both *BCL2* and *MRP1* when compared to untreated or free TAX treated cells. In vivo, the efficacy of the formulation was evaluated in an orthotopic lung cancer model in athymic *nu/nu* mice. The authors compared the organ distribution of non-targeted NLCs labeled with Cy5.5 using in vivo bioluminiscent imagining and magnetic resonance imagining, whereas the internalization of osmium-labeled NLCs by lung cells was studied by electron transmission microscopy in lung tissue sections. A single intravenous or inhaled dose was administered and compared 24 h after administration. It was found that 83% of non-targeted NLC-TAX-siRNA was retained in the lungs, a 3.5-fold higher accumulation when compared to intravenously administered NLCs. These results are in line with Pastor et al who also described no migration of NLCs to other organs after inhaled.[125] LHRH targeting did not significantly influence NLCs distribution within the body. Furthermore, in terms of antitumor efficacy, LHRH-NLC-TAX-siRNA-treated mice exhibited the lowest tumor size at day 24 (2.6 ± 3.0 mm^3, $P < 0.05$ compared to other treatments and initial tumor volume) and half of the animals exhibited bioluminiscence levels of cancer cells below the limit of detection, indicating a disappearance of lung tumor in the experimental disease. Figure 5 illustrates the accumulation of NLCs within the body and the distribution of tumor targeted LHRH-NLCs into the lungs.

A second strategy for DNA and DOX co-delivery within NLCs for lung cancer treatment has been described by Han et al.[123] In this study, transferrin (Tf)-containing ligands were coated onto the surface of NLCs as targeting moieties. In vitro, Tf-NLCs exhibited higher transfection efficiency in A549 cells when compared to Tf-SLNs or non-targeted NLCs. In vivo, Tf-NLCs showed better antitumor efficiency when compared to free DOX or Tf-SLNs, in terms of tumor growth.

These results add to previously reported efficient lipid nanoparticle-based strategies for nucleic acids delivery[133-135] and shed light on the development and mechanism of action of combined chemotherapeutics and nucleic acids-loaded NLCs for lung cancer treatment. The possibility to target these nanocarriers especifically to lung cancer cells should be also highlighted.

Altogether, NLCs represent a promising strategy toward pulmonary delivery as they exhibit good aerosolization properties and good stability of the carrier system and the encapsulated drug when nebulized, and no migration to other organs upon inhalation.[115]

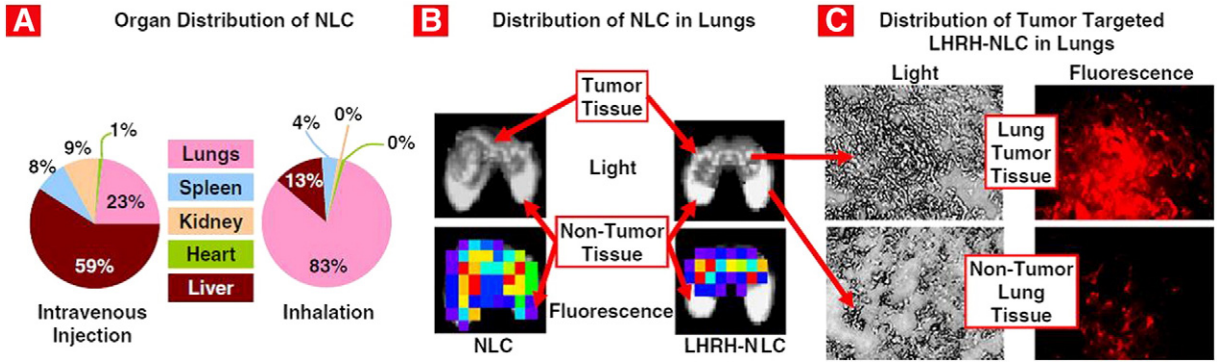

Figure 5. NLC accumulation within the body. **(A)** Body distribution of Cy5.5-labeled NLCs after intravenous administration to mice (left) and after inhalation (right). **(B)** Lung distribution of non-targeted or LHRH-targeted Cy5.5-labeled NLCs in human lung cancer tumor-bearing mice. **(C)** LHRH-NLC accumulation in the lungs of human lung cancer tumor-bearing mice. Red color corresponds to LHRH-NLCs. Reprinted with permission from Elsevier.[122]

Table 6
Ophthalmological applications studied for NLC formulations.

Encapsulated drug	Site of administration	NLC modification	Outcome	Ref.
Cyclosporine A	Topical	Cysteine-polyethylene glycol stearate (Cys-PEG-SA)	Cyclosporine A (CyA) was located in the *cul-de-sac* up to 6 h. Increased precorneal CyA concentration when compared with NLCs without thimoer modification ($P < 0.05$). Non-irritant to rabbit eyes.	146,147
Flurbiprofen	Topical	Chitosan oligosaccharides coating (COS)	COS-coated NLCs increase 7.7-fold flurbiprofen residence time on the cornea when compared to uncoated. Increased transcorneal penetration up to 2.4-fold compared to uncoated NLCs. Corneal biocompatibility according to the ocular irritation test.	148
	Topical	–	Minimal toxicity or irritation according to the Draize test.	149,150
	Topical	Partially deacetylated water-soluble chitosan (PDSC)	Significantly enhanced transcorneal penetration compared to uncoated NLCs and/or flurbiprofen solution. AUC of PDSC-coated NLCs 1.3-fold higher than uncoated NLCs and 2.4-fold of flurbiprofen solution in the cornea. No irritation signs for coated nor uncoated NLCs.	151
	Topical	Carbomer hydrogel embedded	Increased drug release. Non intolerance observed according to the Draize test.	152
Ibuprofen	Topical	–	AUC 4-fold increased in the cornea in comparison with eye drops.	153
Mangiferin	Topical	–	Prolonged drug retention on corneal surface Three months stability of the formulation at 4 °C. Ocular bioavailability increased up to 5.7-fold compared with mangiferin solution.	29
Ofloxacin	Topical	Chitosan oligosaccharide	Penetration rate across rabbit cornea higher when compared with a commercial solution. Improved preocular residence time, controlled drug release and enhanced corneal bioavailability.	154
Triamcinolone acetonide	Topical	–	Delivery of triamcinolone acetonide (TA) to the eye via the corneal and the non-corneal pathway. Encapsulated drug delivered at a constant rate with enhanced accumulation into the scleral tissue, reduced pump efflux and increased residence time.	155-157

Ocular route

The eye is a particularly challenging organ for drug delivery systems. The drug bioavailability is limited by physical barriers that hinder drug access into the eye: (i) muco-aqueous barrier, (ii) corneal epithelium, (iii) iris blood vessels lacking on fenestrations, (iv) non-pigmented layer of the ciliary epithelium and (v) the retinal pigment epithelium (RPE) along with the retinal vessels epithelium.[136] Furthermore, physiological processes like blinking and tear drainage reduce the residence time of ocular drug delivery systems. The use of nanotechnology has been exploited for different ophthalmic applications as the sub-micron size of the nanocarriers allows efficient transport across the ocular barriers.[136-142] Depending on the ocular site of administration (topical, intravitreal, intravenous, transscleral, suprochoroidal or subretinal), differences on the clearance and the toxicity of the nanocarrier are expected. Hence, the nanocarriers should be tailored in accordance to the site of administration, the drug

encapsulated and the disease intended to be treated.[140] Among different nanotechnological approaches, lipid-based nanocarriers have emerged as efficient ophthalmic drug delivery systems, particulartly NLCs, which have been modified to prolong drug retention on the corneal surface encapsulating different drugs.[143-145] Some applications of NLCs to ophthalmology are summarized in Table 6.

In general terms, NLC formulations intended for the ocular route display:

a) Prolonged residence time of the encapsulated drug.
b) Increased ocular bioavailability of the drug.
c) Transcorneal/transscleral drug delivery.
d) Non-toxicity and no irritation in the eye.

Brain drug delivery

Brain drug delivery is probably the most challenging route of administration with more than 98% of newly discovered chemical entities unable to cross the blood brain barrier (BBB).[158] Nanoparticle drug delivery systems have demonstrated to be efficient in overcoming this barrier with several drugs reported to have successfully been transported into the brain using this carrier, including loperamide, dopamine or doxorubicin, among others.[159,160] These drugs could not otherwise indepently permeate the BBB in effective therapeutic concentrations. The mechanisms of transport of nanoparticles across the BBB have been described to occur via receptor-mediated endocytosis.[160,161] Although the use of lipid-based nanoparticles, and solid lipid-based nanoparticles, for brain drug delivery has been widely studied, few studies have evaluated NLCs.[158] Most of the studies regarding NLC formulation have been oriented (i) to optimize the formulation for an increased brain accumulation of the drug and (ii) to evaluate the toxicology of the formulation.[162-164]

Esposito et al[165] compared monoolein acqueous dispersions (MADs) with NLCs as antiparkinsonian drug bromocriptine delivery systems. Both MADs and NLCs efficiently encapsulated BC. However, only NLCs provided prolonged therapeutic effects of bromocriptine in vivo in a 6-hydroxydopamine lesion (6-OHDA) Parkinson model in rats following an intraperitoneal administration. Puglia et al[166] evaluated curcumin-loaded NLCs in CD1 mice and reported that curcumin was able to decrease histone acetylation in the central nervous system (CNS) after intraperitoneal injection when encapsulated within NLCs through an increased curcumin permeation in the CNS.

In spite of these positive results, Beloqui et al[9] evaluated the biodistribution of three different NLC formulations differing on surface charge, particle size and surfactant content following an intravenous administration to rats. All the formulations contained polysorbate 80 and poloxamer 188 as surfactants. The authors described that less than 0.01% of the total injected dose of NLCs was accumulated in the brain, whatever the size or the surface charge. Atlhough it has been described that coating polymeric nanoparticles with surfactants such as polysorbate 80 or poloxamer 188 yields significant pharmacological effects in the central nervous system after intravenous injection into mice and rats,[159,160] it is not the case that NLCs presented increased brain accumulation. These results highlight the need of efficient targeted NLCs toward brain drug delivery following an intravenous administration. There are several examples of successful NLC formulations targeting the brain in the literature. Tsai et al[167] investigated the brain targeting ability of baicalein-loaded NLCs, containing vitamin E and poloxamer 188, via intravenous route. The authors concluded that vitamin E helped increasing baicalein's stability in vivo. Moreover, baicalein-loaded NLCs exhibited a 7.5- and 4.7-fold higher baicalein accumulation in the brain compared to baicalein in solution in the cerebral cortex and brain stem, respectively. Hsu et al[168] also reported that by modulating NLC composition these could successfully target the brain. Compared to an aqueous solution of apomorphine, intravenously injected combined polysorbate 80 and polyethylene glycol (PEG) NLCs greatly improved brain targeting as per in vivo bioluminescence monitoring.

As aforesaid, increased brain accumulation of intravenously injected NLCs has been achieved tailoring NLC formulation. However, there is an unmet need to increase the amount of drug reaching the brain and the 'nose-to-brain' transport appears to be the route of choise in attempt to increase brain drug delivery, as it avoids the BBB and increases drug deposition into the brain.[169] Hence, the current trend is to modify NLCs as per nasal delivery to accomplish increased brain accumulation of the nanoparticles and, thus, increased brain drug delivery. Gabal et al[170] investigated the influence of NLC surface charge on brain delivery through the nasal route and concluded that anionic NLC in situ gel gave the highest drug concentration in the brain, nearly 1.2-fold higher than cationic NLC in situ gel. Devkar et al[171] developed ondansetron hydrochloride-loaded mucoadhesive NLCs for intranasal delivery to the brain and observed that the intranasal route helped to achieve a higher drug targeting (506%) and direct transport percentage (97.14%) to the brain compared to i.v. administration of plain drug. Madane et al[172] also reported an increased curcumin accumulation in the brain when formulated as NLCs after being intranasally administered to rats. More recently, Gartziandia et al[173] demonstrated an efficient brain delivery of chitosan-coated NLCs after being intranasally administered to nude mice.

Although few studies have efficiently delivered NLCs to the brain via nose-to-brain administration, these represent a promising step toward the right direction of attaining increased drug concentration in the brain avoiding the intravenous route.

Future perspectives and conclusions

NLCs are lipid-based nanoparticles containing an unstructured solid lipid core that enables the encapsulation of highly lipophilic drugs, protecting drug from degradation and enhancing their stability. They present many advantages compared to existing nanoparticulated drug delivery systems. They are made of surfactants and lipids that are approved by the FDA and/or EMA and are commercially available in marketed products (Table 1), mainly for oral, dermal and intravenous administration. NLC preparation procedure can be accomplished in the absence of an organic solvent and the process is easily scalable into large batch sizes by high pressure homogenization.

Over the past decade, NLC formulation has undergone a continual improvement in the biomedical field. The 'why now' and the 'how' can be explained by defeated technological barriers hindering the formulation process (e.g. lack of non-diffusing lipophilic dyes) and increased knowledge of the underlying mechanisms of transport of NLCs via different aministration routes (e.g. oral or ocular). Both amendments played a major role in the application of NLCs and achieving succesfull outcomes.

Since being firstly commercialized by Dr. Rimpler Gmb (Germany) for cosmetic purposes, NLCs have been increasingly used for alternative applications and/or diseases than the originally-intented use (e.g. dermatological applications). This has broadened NLC horizons of applicability and has increased the number of studies and publications describing NLCs. Due to decreased particle degradation and prolonged GIT residence times following the oral route, NLCs represent ideal candidates for diseases involving the GIT (e.g. IBDs) when compared to other lipid-based drug delivery systems (e.g. SEDDS).

NLCs have a number of potential industrial applications, mainly due to the many advantages, which have induced the publication of many patents for different applications. However, many of them derive from basic research, and more industrially driven studies are needed. Two recent reviews by Carbone et al[174,175] describe the patenting activity in this field, with a deep critical analysis of the patents comprising NLCs, and the industrial applications for the vectorization of therapeutically relevant molecules, as well as biotechnological products such as proteins and genetic material.

Cutanova Nanorepair Q10 cream was the first NLC containing cosmetical product introduced to the market in October 2005.[176] Another example is FloraGlo®, a viscous solution of the natural antioxidant lutein in safflower oil mixed with a solid lipid and surfactants to produce stable NLC formulations, which showed the ability to ensure a controlled release of lutein and improve its permeability across the skin.[174] Vitamin D derivatives or corticosteroids, such as betametasone and betametasone 14-valerate, are other drugs formulated within NLCs for the treatment of dermal diseases such as atopic dermatitis, acne, psoriasis and skin irritations.[175] In the field of brain drug delivery, a number of patents have dealt with the production and implementation of NLCs as drug carriers.[175]

With regard to the pulmonary route, NLCs present optimal aerosolization properties and good stability of the carrier system and the encapsulated drug when nebulized, accumulating in the lungs and not migrating to other organs upon inhalation. Upon ocular administration, NLCs present prolonged residence time of the encapsulated drug, increased ocular bioavailability of the drug, and non-toxicity and no irritation in the eye. The data describing NLCs administered via nose-to-brain delivery suggest that NLCs can help increase drug concentration in the brain avoiding the intravenous route.

The examples described within the present review through different routes and for different applications clearly illustrate the promise of these nanoparticles for the pharmaceutical market and describes the exciting improvement in this technology in the last decade. Taking into account the increasing number of patented NLC-based formulations and the increasing data available so far, it can be expected that the number of clinical trials pertaining NLCs will substantially increase in the near future. All in all, now more than ever, NLCs appear to be one step closer to their translation into the clinics.[50-69,101,156,157]

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