Lipid nanoparticles: state of the art, new preparation methods and challenges in drug delivery

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Introduction: Nanoparticles are rapidly developing as drug carriers because of their size-dependent properties. Lipid nanoparticles (LNPs) are widely employed in drug delivery because of the biocompatibility of the lipid matrix.
Areas covered: Many different types of LNPs have been engineered in the last 20 years, the most important being solid lipid nanoparticles (SLNs), nanostrucured lipid carriers (NLCs), lipid–drug conjugates (LDCs) and lipid nanocapsules (LNCs). This review gives an overview of LNPs, including their physico-chemical properties and pharmacological uses. Moreover, it highlights the most important innovations in the preparation techniques of LNPs, aimed to encapsulate different molecules within the lipid matrix. Finally, it gives a short perspective on the challenges of drug delivery, which are a potential field of application for LNPs: cancer therapy, overcoming the blood–brain barrier and gene and protein delivery.
Expert opinion: LNPs are a safe and versatile vehicles for drug and active delivery, suitable for different administration routes. New technologies have been developed for LNP preparation and studies are currently underway in order to obtain the encapsulation of different drugs and to deliver the active molecule to the site of action.

Keywords: administration routes, drug encapsulation, LDC, LNC, NLC, physico-chemical stability, polymorphism, preparation techniques, SLN, therapeutic challenges

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1. Introduction

Rapid advances in the ability to produce nanoparticles of uniform size, shape and composition have caused a revolution in the pharmaceutical sciences. Due to their size-dependent properties, nanoparticles offer the possibility of developing new therapeutics. The ability to incorporate drugs into nanocarriers offers a new prototype in drug delivery that can be used for many therapeutic goals.

Owing to lipid biocompatibility and versatility, lipid nanoparticles (LNPs) showed many advantages over polymeric nanoparticles, and have been widely used for drug and active delivery [1]. Apart from liposomes and niosomes, which are vescicular nanostructures made up of phospholipids and amphipatic polar lipids, respectively, with a long and safe history of use, in the last two decades many nanoparticulate formulations have been engineered in the form of nanospheres and nanocapsules by using solid and liquid lipids as matrices. One fundamental advantage of LNPs with regard to other lipid colloidal drug delivery systems (liposomes, niosomes, etc.) and to nanoemulsions, is their great kinetic stability and rigid morphology. Nanoparticles can be divided into two main families: nanospheres, which have a homogeneous structure in the whole particle, and nanocapsules, which exhibit a typical core-shell structure [2]. Main advantages of lipid carriers over other traditional drug carriers are good biocompatibility, lower cytotoxicity, good production scalability, the modulation of drug release, the avoidance of organic

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Article highlights.

- Lipid nanoparticles (LNPs) are a safe vehicle for drug delivery, made of physiological or physiologically related lipids.
- Many different types of LNPs have been engineered, the most important being solid lipid nanoparticles (SLNs), nanostrucured lipid carriers (NLCs), lipid–drug conjugates (LDCs) and lipid nanocapsules (LNCs).
- High-pressure homogenization (HPH) is a well-established technique, but new technologies are currently emerging for LNP preparation.
- LNPs are easy to scale up, even if some process parameters can negatively influence their stability over time.
- LNPs can be administered by various routes according to different therapeutic target.
- Anticancer therapy, overcoming of the blood–brain barrier (BBB), protein and gene delivery are the main emerging topics nowadays for LNP applications.

This box summarizes key points contained in the article.

solvents in the preparation process and a wide potential application spectrum (oral, dermal, intravenous, etc.). In recent years, many preparation methods for LNPs have been developed in order to comply with the need of encapsulating more and more complex molecules [3].

2. SLNs, NLCs, LDCs, LNCs

The most known formulation among LNPs is solid lipid nanoparticles (SLNs). SLNs are nanospheres made from solid lipids with a mean photon correlation spectroscopy (PCS) diameter between approximately 50 and 1000 nm.

They are produced mainly according to hot homogenization method, which generally implies the use of high-pressure homogenization (HPH) [1]. The SLN production is based on solidified nanoemulsion technologies. HPH, high shear homogenization [4] and ultrasonication [5] are all used for nanoemulsion preparation.

Nanostrucured lipid carriers (NLCs) are LNPs characterized by a solid lipid core consisting of a mixture of solid and liquid lipids: the resulting matrix of the lipid particles shows a melting point depression compared with the original solid lipid, but the matrix is still solid at body temperature. Depending on the method of production and on the lipid blend composition, different types of NLCs are obtained: imperfect, amorphous and multiple type. In the imperfect type, lipid crystallization is altered by small amounts of oils. In the amorphous type, the lipid matrix is solid but not crystalline (amorphous state): this can be achieved by mixing special lipids, for example, hydroxyoctacosanyl hydroxystearate with isopropyl myristate. In the multiple type, the solid lipid matrix contains tiny oil compartments: they are obtained by mixing a solid lipid with a higher amount of oil. The basic idea is that by giving a certain nanostructure to the lipid matrix, the payload for active compounds is increased and expulsion of the compound during storage is avoided. NLCs can be produced by HPH and the process can be modified to yield lipid particle dispersions with solid contents from 30 to 80% [6].

To overcome the limitation of low drug loading capacity of SLNs and NLCs for hydrophilic drugs, lipid–drug conjugates (LDCs) nanoparticles were developed. In a typical process, an insoluble drug–lipid conjugate bulk is first prepared either by salt formation (e.g., with a fatty acid) or by covalent linking (e.g., esters or ethers). The obtained LDC is then processed with an aqueous surfactant solution to a nanoparticle formulation using HPH [7].

Another kind of LNP is lipid nanocapsules (LNCs), organized in an internal liquid or semi-liquid oil core and an external lipid layer solid at room temperature [8]. LNCs have nearly the same particle size as SLNs, but they have a core-shell structure. The phase inversion temperature (PIT) method proposed by Shinoda and Saito for the preparation of nanoemulsions was modified for LNC production [9]. The PIT concept uses the specific ability of some polyethoxylated surfactants to modify their affinities for water and oil as a function of the temperature. In the PIT nanoemulsion preparation method, the use of such surfactant type leads to an emulsion inversion from oil-in-water (o/w) macroemulsion to a water-in-oil (w/o) emulsion when temperature is increased above the PIT, and to the formation of an o/w nanoemulsion below the PIT. For the preparation of the LNCs by PIT method three main components are used: an oil phase, an aqueous phase and a non-ionic surfactant. All the components are mixed under magnetic stirring and heated from room temperature up to a temperature T2, above the PIT, to obtain a W/O emulsion. This is followed by a cooling process to a temperature T1, below the PIT, leading to the formation of an O/W emulsion. Several temperature cycles crossing the phase inversion zone (PIZ) between T2 and T1 are then carried out, in order to reduce the droplet size of the obtained emulsion. The use of variable operating temperatures (T1/T2) allows the formation of an O/W nanoemulsion with reduced droplet size. Then the system is rapidly cooled to room temperature in order to precipitate the LNCs.

3. Preparation techniques

Since the 50s, HPH has been proved to be a simple technique, well established on large scale, for the production of o/w parenteral emulsions and already available in pharmaceutical industry. Recently, it has been applied for SLN, NLC and LDC production and represents the main method established for these nanoparticles. However, this method involves some critical process parameters, like high temperatures, high pressures (cavitation force), that may cause significant thermodynamic and mechanic stress for the resulting product. For this reason, and in order to overcome patented methods,

suitable alternative and easy handling production methods for LNP preparation have been deeply investigated.

SLNs can be produced starting from microemulsion templates. Gasco [10] was the first researcher to patent a microemulsion template for SLN preparation. According to the invention, lipids are heated above their melting point and an aqueous phase containing surfactants and co-surfactants is added under stirring at the same temperature to form a clear O/W microemulsion. Multiple W/O/W can be prepared, too. The microemulsion is then diluted in cool water (2 – 10°C), in order to precipitate the SLNs with reduced mean particle size and narrow size distribution.

A few years ago, some researchers [11] developed another microemulsion-based method to produce stable SLNs. The authors started from an O/W microemulsion, consisting of an emulsifying wax as lipid phase and a polymeric surfactant solution as water phase, kept at a temperature of 37 – 55°C, according to the melting point of the emulsifying wax. SLNs were obtained by cooling the undiluted O/W microemulsion at room temperature while stirring. An advantage of this invention is that SLNs can be formulated at mild operating temperatures – rapidly, reproducibly and cost-effectively – from the microemulsion precursor in a one-step process and contained in only one manufacturing vessel, vial or container.

Solvent-based methods have been proposed to encapsulate molecules with stability and bioavailability problems, despite the fact that toxicological issues of the solvent are a limiting aspect. One of the main advantages of solvent-based methods is the mild operating temperature, which can be useful for encapsulation of thermosensitive drugs.

Among them, solvent injection (or solvent displacement) is the simplest one and is based on dissolving the lipid in a water miscible organic solvent (ethanol, acetone, isopropanol) and injecting this solution through a syringe needle in water under stirring with the lipid precipitating in the form of nanoparticles on contact with water [12,13].

Alternative solvent-based methods start from an emulsion precursor: O/W or W/O/W emulsions can be prepared by using either a volatile or a partially water miscible organic solvent, which dissolves the lipid. Nanoparticles are formed when the solvent is removed either by evaporation (solvent evaporation method) [14,15] or by water dilution (solvent diffusion method) [16,17].

Recently, a new method was developed to prepare, in a controlled way, SLNs by coacervation, starting from fatty acids alkaline salts, allowing the incorporation of drugs, also if thermosensitive, without using very complex equipment or dangerous solvents, and which is, therefore, inexpensive for laboratory and industrial application [18]. This method is based on a slow interaction between a micellar solution of a sodium salt of a fatty acid and an acid solution (coacervating solution) in the presence of a proper amphiphilic polymeric stabilizing agent. By lowering the pH, the nanoparticles can be precipitated.

Supercritical fluid (SCF) technology has gained increasing interest in the last years for nanoparticle production. SCF is obtained above its critical pressure and temperature: above this fluid's critical point, the solubility of a substance in the fluid can be modulated by a relatively small change in pressure. Due to its low critical point at 31°C and 74 bar, and its low cost and non-toxicity, carbon dioxide is the most widely used SCF. Two main SCF-based methods have been developed for SLNs production: SCF extraction of emulsions (SFEE) and gas-assisted melting atomization (GAMA).

SFEE is based on a simple principle, whereby the lipid nanosuspensions are produced by SCF extraction of the organic solvent from O/W emulsions [19]. O/W emulsions are introduced into an extraction column from the top and simultaneously, supercritical CO_2 is introduced countercurrently from the bottom. When the o/w emulsion containing the lipid and the drug is introduced into the supercritical CO_2 phase, solvent extraction into the supercritical CO_2 phase occurs, leading to precipitation of lipid–drug material dissolved in the organic phase as composite particles. One of the advantages of this technique is that the solvent extraction efficiency using supercritical CO_2 is much higher than for the conventional methods, such as solvent evaporation, liquid extraction and dilution, allowing a fast and complete removal of the solvent and a more uniform particle size distribution.

In GAMA method, lipids are placed in a thermostated mixing chamber (CM), where they are melted and kept in contact with supercritical CO_2 at selected temperature and pressure conditions. Then, the lipid-saturated mixture is forced through a nozzle by opening the valve at the bottom of the CM: the rapid depressurization of the mixture creates a high degree of supersaturation and the precipitation of microparticles, which are collected by a collection system and dispersed in water by vortexing and by ultrasound treatment, in order to obtain suspensions [20].

Another method has been developed for producing SLNs, by using a membrane contactor [21]: a proper module has been realized, including a ceramic membrane (0.1, 0.2, 0.45 μm pore size), which separates the water phase, allowed to circulate tangentially to the membrane surface, and the lipid phase. The lipid phase is heated in a pressurized vessel above its melting point, conveyed through a tube to the module and pressed through the membrane pores, allowing the formation of small droplets, which are detached from the membrane pores by tangential water flow. SLNs are formed after cooling of the obtained water dispersion.

Lipid particles can also be formed in solid powdered state, owing to different techniques [22]: in these cases, LNPs and lipid microparticles (LMPs) can be obtained, according to the method and operative conditions used. The composition of LMPs is equivalent to LNPs, but they are in the micrometer size range (mean diameter > 1 μm). Given the similar composition between LMPs and LNPs, LMPs can be considered as physiologically compatible, physico-chemically stable and allowing a large-scale production. The difference between

LMPs and LNPs lies in their respective size ranges, meaning that their application domains and administration routes can be different [23].

Spray-drying is a one-step process which converts a liquid feed to a dried particulate form: in the case of lipid particles, the feed is an organic solvent solution, which is first atomized to a spray form that is put immediately into thermal contact with a hot gas, resulting in the rapid evaporation of the solvent to form dried solid particles. The dried particles are then separated from the gas by means of a cyclone, an electrostatic precipitator or a bag filter [24].

In cryogenic micronization, lipid matrices, obtained either by melt dispersion (the drug is mixed in a molten lipid) or solvent stripping (the drug and lipid are dissolved into a solvent mixture under stirring, e.g., benzyl alcohol, ethanol), are stored at -80°C and then micronized in a customized apparatus supplying liquid nitrogen during the process. This technique can be used only for the production of microparticles of 5 – 5000 µm in diameter [25].

In the spray-congealing method, lipids are heated to a temperature above their melting point. The hot lipid is atomized through a pneumatic nozzle into a vessel which is stored in a carbon dioxide ice bath or at room temperature. The microparticles (50 – 500 microns) obtained are then vacuum dried at room temperature for several hours [26].

In the electrospray technique, the electrostatic atomizer comprises a nozzle connected to a high-voltage power supply and is supplied with a liquid to be atomized. The lipid solution in organic solvent is contained in a syringe, with a metal capillary connected to a high-voltage power supply as one electrode. A metal foil collector is placed opposite the capillary as a counter electrode. Depending on the properties of the liquid, the flow rate and the voltage applied can be modulated, and droplets can be produced with a close size distribution and nano- or micrometer size range. Solid lipid particles can be formed by evaporating the solvent from the droplets produced traveling through the electrical field [27].

In **Table 1**, various types of lipid particles and the relative preparation methods are summarized.

4. Drug encapsulation

Drug can be encapsulated in LNPs in different ways, according to the preparation method employed. However, in every preparation technique an interaction occurs between drug and lipid which leads to the encapsulation phenomenon. Drug encapsulation can be evaluated through two main parameters, drug loading and drug encapsulation efficiency, where the former is the ratio between drug and lipid in nanoparticles and the latter is the ratio between the drug recovered in nanoparticles and the amount weighted for the preparation of the same.

For an objective evaluation of these two parameters in a LNP system, separation of nanoparticles from the outer phase should be performed, preferably through centrifugation.

In particular, drug encapsulation has been deeply studied in SLNs. Drug can be distributed in the lipid matrix in different ways: into a homogeneous matrix, into nanoparticles shell and as a lipid-coated core [1]. In the homogeneous matrix model, the drug is molecularly dispersed or is present in amorphous clusters in the lipid matrix. The drug-enriched shell-type contains an outer shell enriched with drug, which covers a lipid core. It is formed mainly when phase separation occurs between drug and lipid, during the cooling process in hot HPH. The drug-enriched core-type forms when the drug starts to precipitate before the lipid, and the lipid shell forms around this core. It is formed mainly when the drug concentration is close to its saturation solubility in the melted lipid in hot HPH.

The shell-enriched model implies that an important amount of drug is exposed on the surface of the particles, and is responsible for burst release phenomenon. As a consequence, a great part of the drug is not really encapsulated in the lipid matrix, but absorbed on its surface. In fact, a proper evaluation of drug encapsulation should involve not only the centrifugation of nanoparticles, but also the washing of the same with a proper solution, suitable for removing the drug adsorbed in the outer shell, before determining the drug encapsulation efficiency [28,29]; also *in vitro* drug release should be studied to determine the extent of drug burst release.

Dominant factors influencing the release profiles from SLNs are the production parameters: surfactant concentration and operating temperature [1]. During particle production by the hot homogenization technique, drug partitions from the liquid oil phase to the aqueous phase. The higher the operating temperature and surfactant concentration, the greater is the saturation solubility of the drug in the water phase. During the cooling process, the solubility of the drug in the water phase decreases continuously with decreasing temperature of the water phase, which means a re-partitioning of the drug into the lipid phase occurs. When reaching the recrystallization temperature of the lipid, a solid lipid core starts forming including the drug which is present at this temperature in this lipid phase. The already crystallized lipid core is not accessible anymore for the drug, consequently the drug concentrates on the surface of the particles. The amount of drug in the outer shell and on the particle surface is released in the form of a burst, the drug incorporated into the particle core is released in a prolonged way. Therefore, the extent of prolonged and burst release can be controlled via the solubility of the drug in the water phase during production, which means via the operating temperature and the surfactant concentration used, with higher operating temperatures and higher surfactant concentrations increasing the burst release [1].

Both drug encapsulation and drug loading depend on the solubility of the drug in melted lipid, the miscibility of drug melt and lipid melt, the chemical and physical structure of solid lipid matrix (water solubility, partition coefficient) and the polymorphic state of lipid material [1].

Briefly, drugs can be divided in hydrophilic and hydrophobic, according to their water solubility and

Table 1. Lipid particles preparation methods.

Method	LNP suspension			Method	LNPs/LMPs in powdered form		
	Ref.	Year	Type		Ref.	Year	Type
HPH	[93]	1996	SLNs/NLCs/LDCs	Spray-drying	[94]	2006	SLNs/LMPs
Ultrasonication	[5]	2003	SLNs	Spray-congealing	[95]	1996	LMPs
High shear homogenization	[4]	2005	SLNs	Electrospray	[27]	2010	SLNs/LMPs
Solvent injection	[13]	2002	SLNs	GAMA	[20]	2009	SLNs
Solvent evaporation	[14]	1996	SLNs	Cryogenic micronization	[96]	2004	LMPs
Solvent diffusion	[16]	2003	SLNs				
Microemulsion dilution	[10]	1993	SLNs				
Microemulsion cooling	[97]	2001	SLNs				
Coacervation	[98]	2008	SLNs				
PIT	[99]	2001	LNCs				
SFEE	[100]	2004	SLNs				
GAMA	[20]	2009	SLNs				
Membrane contactor	[101]	2007	SLNs				

GAMA: Gas-assisted melting atomization; HPH: High-pressure homogenization; LDCs: Lipid–drug conjugates; LMPs: Lipid microparticles; LNPs: Lipid nanoparticles; NLCs: Nanostrucured lipid carriers; PIT: Phase inversion temperature; SFEE: Supercritical fluid extraction of emulsions; SLNs: Solid lipid nanoparticles.

partition coefficient. For the former class, suitable strategies have to be adopted to reduce partitioning of the drug into the outer water phase and enhance drug encapsulation [30]. Among them, the most important are:

1) starting from a template which allows the encapsulation of hydrophilic drugs: that is, W/O/W emulsion with organic solvent [14,16] or W/O/W microemulsion [31]. O/W emulsion with partially water miscible organic solvents [28]
2) using the hydrophobic ion pairing (HIP) technique: it can be performed between some charged hydrophilic drugs and opposite charged surfactants, in order to decrease the drug water solubility and enhance the drug apparent partition coefficient, allowing enhancement of drug encapsulation within nanoparticles [29,32,33]
3) preparing LDC nanoparticles [7,34]
4) using polymer lipid hybrid nanoparticles (PLNs): a complex between drug and ionic polymer is formed by neutralizing charges on drug with polymer counter ion, and the formed complex is encapsulated into nanoparticles [35-38]
5) using water-free preparation methods (i.e., electrospray) [39]

For hydrophobic drugs, instead, insufficient drug loading or burst effect can be improved by the use of NLC formulation: NLCs allow a higher drug load due to the formation of a less ordered lipid matrix, improving also release properties [6].

5. Scale up and stability issues

LNP production can be easily scaled up, owing to the adopted preparation method. However, many stability problems can be associated with LNPs and can be an obstacle during the scale-up process.

First of all, polymorphism has to be taken into account. According to Siekmann and Westesen [40], the melting point decrease of LNP colloidal systems can be due to the colloidal sizes of the particles, in particular to their high surface-to-volume ratio, and not to recrystallization of the lipid matrices in a metastable polymorph. If the bulk matrix material is turned into LNPs, the melting point is depressed [41]. The presence of impurities, surfactants and stabilizers could also affect this phenomenon [42,43]. However, polymorphism can also be present on LNP preparation, according to the method and to the lipid matrix used: in this case differences of 10 – 20°C between the raw material and the nanoparticles melting point can be tolerated. For instance, fatty acids and triglycerides showed polymorphism on coacervation and hot homogenization method, respectively [44,18]. In the case of spray-drying, unstable polymorphic forms were obtained due to rapid solvent evaporation. The same consequence was observed with the spray-congealing process of micropellets [45]. Consequently, nanoparticles prepared from triglycerides which are solid at room temperature did not necessarily crystallize on cooling to common storage temperatures. The particles can remain liquid for several months without crystallization (supercooled melt) [46]. Moreover, for triglycerides the α and β_0 forms have the tendency to be transformed to a form with better chain packing such as the β form, which is the most stable and high melting. These unstable forms gradually transform toward the most stable form during storage, losing the initial nanoparticle spherical surface structures, leading to crystalline aggregate growth and causing drug leakage, owing to a reduction of amorphous regions in the carrier lattice [47].

Second, sterilization, when needed, is a critical process parameter for LNP scale up and stability [48]: γ-irradiation is

a current sterilization technique for pharmaceutical products. However, chemical degradation of the lipids can take place during irradiation: ionizing radiation has consequently been excluded or at least more studies will be necessary, before it will be accepted as a safe and convenient sterilization technique. In various studies, an autoclaving approach was preferred because it did not change Zeta potential and mean size of the particles, even if this is paradoxical considering the influence that temperature can have on nanoparticles stability. Sterile filtration can be applied only for particles with size lower than the filter pores and has not been deeply studied for LNP suspension.

Another important instability mechanism is the phase separation [49], due to the aggregation of particles that can be reversible (flocculation) or irreversible (coalescence, sedimentation). Also gelling phenomena can happen during storage. In order to overcome these problems, proper surfactants can be used: they can stabilize LNP suspension according to electrostatic repulsion, which increases Zeta potential (anionic or cationic surfactants), or they can act as steric stabilizers (non-ionic surfactants). It should be noticed, however, that surface stabilization, especially electrostatic stabilization and Zeta potential, are very sensitive to pH and electrolytes eventually present in the outer phase, which can cause destabilization of suspension.

The obtainment of solid forms, from which water has been eliminated, is an effective strategy to overcome the problems regarding storage stability of LNPs. This can be obtained by spray-drying or lyophilization of the suspension, but in the practice many important parameters have to be considered to obtain re-dispersible powders after these processes. Spray-drying causes coalescence of the particles, consequent to melting of the lipid at the high temperatures used. The addition of carbohydrates to the suspension prior to spray-drying can reduce coalescence, and the use of mixtures of ethanol and water for the evaporation step can allow the reduction of the operative temperature [50]. In the lyophilization process, particle aggregation takes place easily. For this reason, the use of sugars as cryoprotectants before the freezing step is highly recommended [51]. Interestingly, for SLNs prepared by coacervation, no particle aggregation occurred after freeze-drying and re-dispersible powders can be obtained because of the presence of the polymeric stabilizers, which act like cryoprotectants themselves [18].

Stability of LNPs has been investigated also in biological fluids, in particular in gastrointestinal fluids and in serum.

In gastrointestinal fluids, instability can occur following particle aggregation or enzymatic degradation of the lipid matrix.

In the gastrointestinal tract (GIT), LNP stability to aggregation, due to ionic strength and acid pH of the stomach, can be increased by optimizing the surfactant mixture. Prerequisites for stability were identified in minimum 8 – 9 mV Zeta potential in combination with steric stabilization [52].

Enzymatic degradation is due to the pancreatic lipase: degradation velocity is substantially affected by the length of the fatty acid chains in the triglycerides and from the surfactants used. The longer the fatty acid chains in the glycerides, the slower the degradation. The influence of surfactants can be degradation accelerating (e.g., cholic acid sodium salt) or a hindering, degradation slowing down effect, due to steric stabilization (e.g., Poloxamer 407) [53].

The interaction of LNPs with the major circulatory protein, serum albumin, has been investigated recently. By photo correlation spectroscopy and atomic force microscopy, albumin adsorption on the particle surface was demonstrated, forming a capping layer of 17 nm and increasing the size of tested particle populations only slightly [54]. Various research groups have also increasingly focused on improving their stability in body fluids after administration by coating of LNPs with hydrophilic molecules such as poly(ethylene)glycol (PEG) derivatives. Analogously to polymeric nanoparticles and liposomes, when LNPs are coated with PEG, their surface hydrophobicity is favorably modified and LNPs are sterically stabilized, thus suppressing the binding of serum proteins and other opsonic factors. Coating of LNPs with PEG increases stability and plasma half-life of LNPs in order to decrease phagocytic uptake, and therefore improves the biovailability of drugs [55].

6. Administration routes

LNPs are composed of physiological or physiologically related lipids: therefore, pathways for transport and metabolism are present in the body which may contribute to a large extent to the *in vivo* fate of the carrier.

Topical administration is an area of great potential for LNPs and with a short time-to-market, especially for cosmetic formulations. Distinct advantages of LNPs in topical drug delivery are the ability to protect chemically labile ingredients against chemical decomposition, the possibility to modulate drug release and the property of forming adhesive lipid films onto the skin that can have an occlusive effect [56].

Basically, LNPs can be used for all parenteral applications, ranging from intra-articular to intra-muscular, subcutaneous and intravenous administration, owing to particle size and therapeutic goal [54]. Because of their small size, LNPs may be injected intravenously and used to target drugs to particular organs. The particles, as with all intravenously injected colloidal particulates, are cleared from the circulation by the liver and spleen. LNPs able to avoid the reticuloendothelial system (stealth) may be obtained by using PEG.

Oral administration of LNPs is possible as aqueous dispersion or alternatively after transformation into a traditional dosage form, that is, tablets, pellets, capsules or powders in cachets. For the production of tablets, the aqueous LNP dispersion can be used instead of a granulation fluid in the granulation process. Alternatively, LNPs can be transferred to a powder (e.g., by spray-drying or freeze-drying) and added to the tabletting powder mixture. LNP powders can be used for the filling of hard gelatin capsules. Cachets are also

possible using spray-dried or lyophilized powders. For cost reasons, spray-drying might be the preferred method for transferring LNP dispersions into powders [1].

LNPs can help drug solubilization in the GIT, because of their ability to retain a poorly soluble substance in a solubilized state and to enhance solute–solvent interactions, also after mixing with endogenous solubilizers, such as bile acids or phospholipids. Moreover, the protective effect of LNPs, coupled with their sustained/controlled release properties, prevents drugs/macromolecules from premature degradation and improves their stability in the GIT [57]. Their nanoparticulate state facilitates their uptake by M cells of Peyer's patches, which in turn enables the carrier system to bypass the effect of first-pass metabolism, through lymphatic absorption. The reduction of side effects (i.e., stomach toxicity of non-steroidal anti-inflammatory drugs (NSAIDs)) and masking of taste are also two relevant goals for oral administration of LNPs [58].

LNPs are considered as a promising drug carrier system for pulmonary administration, even if they have been rather unexploited so far [59,60,24,19]. However, a preliminary *in vivo* tolerance study has been carried out on rats with LMPs composed of glyceryl behenate as a lipid matrix and poloxamer 188 (Lutrol® F68) as a surfactant, administered intratracheally. Results did not show significant differences between placebo groups and microparticles-treated rats. It has been concluded that the studied lipid particles seem to be well tolerated by the lower airways, but tolerance must still be assessed after repeated administration [61].

In recent years, LNPs have been exploited for ocular delivery [62-64], especially positively charged nanoparticles [65-67], which can enhance corneal bio-adhesion and drug permeation, according to various mechanism, including phagocytosis by cornea epithelial cells.

Also rectal route has been proposed for LNP formulation [68,69].

7. Therapeutic challenges

LNPs have been proposed for delivery of several drugs and activities for various objectives. The examination of all the applications of LNPs overcomes the purpose of this review. However, among the most intriguing and recent tasks for LNPs, there are some challenges that are becoming the hot topic of drug therapy nowadays. Anticancer therapy, the overcoming of the blood–brain barrier (BBB), protein and gene delivery are research fields where the need of a safe and versatile drug carrier is imperative, and LNPs have been proposed and tested to these goals.

7.1 Cancer therapy

The rationale of using LNPs for anticancer drug delivery is based on some physiological mechanisms [70].

A tumor is often associated with a defective, leaky vascularization as a result of the poorly regulated nature of tumor angiogenesis. In addition, the interstitial fluid within a tumor is usually inadequately drained by a poorly formed lymphatic system. As a result, submicron-sized particulate matter may preferentially extravasate into the tumor and be retained there. This is often referred as the 'enhanced permeability and retention' (EPR) effect [71]. This EPR effect can be taken advantage of by a properly designed nanoparticle system to achieve passive tumor targeting. But, following intravenous administration, drug delivery systems such as polymeric nanoparticles are rapidly cleared from the systemic circulation by the mononuclear phagocyte system (MPS). Reduced particle size, natural composition and, more importantly, hydrophilic surface (coating with hydrophilic polymers) are necessary to avoid the opsonization of the complement in plasma and the consequent elimination by the MPS [72]. This type of polymer-coated drug delivery system is often referred to as 'long-circulating' drug carriers. The use of long-circulating LNPs is at an early stage, but interest in its use is increasing, due to the lower toxicity of lipid matrix compared with polymeric one [8,33,70,73-75].

In order to increase cancer cell-selective cytotoxicity, a strategy that is gaining attention is to surface-engineer nanoparticles for active targeting. This strategy exploits the differences between cancer cells and healthy cells, in particular surface antigen differences. Ideally, the antigen that will allow active targeting is expressed exclusively on cancer cells, is an integral part of an essential cellular function of the cancer cells, and does not easily mutate as the cancer cells proliferate. Various types of receptors such as lipoproteins, folate, different peptide receptors, growth factor receptors and transferring receptor overexpress on the surface of malignant tissues compared with normal tissues. Few preliminary examples are present in literature about active targeting of LNPs: in particular targeting through folate, transferrin and lectin receptors have been proposed [76-79].

Another important limitation of anticancer drug therapy is multidrug resistance (MDR), which is mainly associated with P-glycoprotein (P-gp)-mediated cellular efflux system. P-gp is a 170 kDa transmembrane protein member of the ABC (ATP-binding cassette) family, which acts as an efflux pump from the cell for many drugs (anticancer agents, antibiotics, etc.). The efficiency of many drugs (especially anticancer drugs) is dramatically reduced by the P-gp efflux pump, being one of the main factors leading to drug resistance *in vivo*. Many compounds with P-gp inhibitory activity have been identified or synthesized: they are sometimes referred as 'chemosensitizers' because by inhibiting P-gp-mediated cellular efflux of the cytotoxic drugs, they apparently restore the sensitivity of the drug-resistant cells to the chemotherapeutic treatment. However, chemosensitizers led to significant toxicities and pharmacokinetic interactions with the co-administered cytotoxic drug [70]. LNPs can help in overcoming the MDR phenomenon, probably because they carry the encapsulated drug into the cancer cells by endocytosis, thereby bypassing the P-gp drug efflux mechanism [80-82].

It is also reported that some components of the LNP formulations can act directly as inhibitors of P-gp [83].

7.2 Overcoming the BBB

The BBB acts as a physical barrier and regulates the passage of selected molecules between the bloodstream and the brain by either paracellular or transcellular pathways. Owing to the presence of the tight junctions between the endothelial cells, the passive diffusion of solutes through the paracellular pathway is very limited. Specific transporters (with activities regulated by the brain's metabolic needs), however, facilitate the brain uptake by active transport of many nutrients, vitamins and hormones: among them some endogenous proteins. At the same time, molecules are continuously eliminated from the brain by the same efflux transporters (ABC transporters), that are involved in MDR phenomenon [84].

The main targeting strategies for the brain are the modulation of efflux transporters (since LNPs could overcome P-gp excretion also at the level of the BBB) [85] and the biological active targeting which can be obtained by endogeneous transporters and peptide conjugation [86,87]. Among the most important protein used to target the brain there are apolipoproteins, especially ApoE, which are involved in the mechanism of lipoproteins uptake by the brain and in the brain lipid metabolism. It was recently reported that SLNs formulations using polysorbates as stabilizers, showed adsorption of ApoE and, consequently, due to ApoE adsorbed on the surface, SLNs accumulation in the brain might occur [88].

7.3 Protein and peptide delivery

Therapeutic application of peptides and proteins is restricted by their high molecular weight, hydrophilic character and limited chemical stability, which cause low bioavailability, poor transfer across biological membranes and low stability in the bloodstream. Most of the available peptides and proteins are delivered by injection, but their short half-life demands repeated doses that are costly, painful and not well tolerated by patients. In recent years, major efforts are being directed toward the production of needle-free alternatives to administrate these biomacromolecules mainly by oral route, but not exclusively; other administration routes, such as buccal, nasal, pulmonary or transdermal have also been studied. LNPs could be useful for peptide and protein delivery due to the stabilizing effect of lipids and to the absorption promoting effect of the lipidic material that constitute this kind of nanoparticles [89].

Moreover, for a long time particulate carriers have been sought as vehicles for protein antigens. An extensive work has been developed in the area of vaccine formulation using various biodegradable polymeric nanoparticles and liposomes, since most peptide or protein antigens are ineffective for mucosal immunization due to proteolytic degradation at mucosal sites. LNPs can be useful as adjuvant formulations for vaccination with either protein antigens or nucleic acids. Although still sparse, the existing information clearly indicates that, as for biodegradable microspheres, LMPs act as effective vaccine carriers with immunoadjuvant properties by parenteral and mucosal routes [90].

7.4 Gene delivery

Gene therapy is a rapidly advancing field with great potential for the treatment of genetic and acquired systemic diseases as well as for vaccination. It can be achieved by introducing genetic material (plasmid DNA; pDNA) into target cells to enhance or correct protein expression or, alternatively, by using antisense oligonucleotides (ASO) or short interfering RNA (siRNA) as transcription and/or translation inhibitors to silence defective genes [91].

Nucleic acids are hydrophilic negatively charged macromolecules, very labile in the biological fluids: systemic administration of naked nucleic acids does not result in effective therapeutic responses. Then, the interaction between active molecules and biological membranes is necessary to initiate the entrance into cells, but this process is not spontaneous because the negatively charged surface of both nucleic acids and cell membranes hampers the interaction, and nucleic acid hydrophilic character prevents the passing through lipophilic cell membranes. LNPs can protect nucleic acids from digestion in biological fluids and have shown to enter into cells by endocytosis [89].

The use of LNPs in gene therapy requires positively charged surface to bind electrostatically nucleic acids, leading generally to an excess of positive charges in the final complexes [92].

8. Expert opinion

LNPs gained their own importance in the nanoparticles field owing to the biocompatibility of the lipid matrix. In fact, one of the most important issues for pharmaceutical and cosmetic industry is the safety of the material, which has to meet the demands of the regulatory authorities. SLNs are the most important type of LNPs and are prepared mainly by using HPH, which is a well-established technique; however, the disadvantages related to this production method (high operating temperatures, cavitation forces), as well as the need of encapsulating many types of drugs, with different physico-chemical features and various stability and solubility problems, led to the development of new types of LNPs (like NLCs, LDCs, LNCs) and of innovative preparation methods. Currently, many different strategies have been employed to facilitate the incorporation of different drugs and actives into LNPs, according to their different chemical nature. In fact, drug encapsulation efficiency and drug loading are two key parameters for the evaluation of LNPs as drug delivery system. A high encapsulation efficiency stands for an 'economic' loading process where the major part of the drug is effectively loaded into nanoparticles; drug loading is important since the amount of drug loaded into nanoparticles should be in its therapeutic range in order to achieve therapeutic efficacy.

LNPs proved to be easy to scale up, even if some process parameters are still critical and can negatively influence their stability over time; suitable strategies can be adopted in order to overcome these problems.

LNPs can be administered by various routes, according to the therapeutic target, and, since they are composed of physiological or physiologically related lipids, their *in vivo* fate depends on the pathways for transport and metabolism present in the body.

Moreover, many different applications have been exploited and patented recently for LNPs: among these the hot topic of drug therapy nowadays. In particular, the research in the field of LNPs is directed both toward the encapsulation of macromolecules (nucleic acids and proteins) – to avoid enzymatic degradation and increase transfer across biological membranes – and toward the passive and active drug targeting to specific sites of action (tumor, brain) – to increase the efficacy and reduce the toxicity of drug therapy.

In future perspective, the formulation strategy of LNPs will be strictly connected to the goals of the most important topic in drug delivery, that is, the formulation method will be adapted to the encapsulation of more and more complex drugs, and to functionalize the nanoparticles in order to deliver the molecule within the site of action. Moreover, despite the fact that LNPs are well established as a safe drug delivery system, it is important to respond to the possible toxicological concerns of the new emerging methods of preparation. On the other side, further work is needed to study the interaction of LNPs with their biological surrounding to deeply understand their mechanism of action at a cellular level, and consequently their future possible application in drug therapy.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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