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Development and tailoring of hybrid lipid nanocarriers

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Abstract: Lipid nanoparticles are considered one of the most promising systems for controlled release of therapeutic molecules highly hydrophobic and with low biodisponibility. Solid lipid nanoparticles and nanostructured lipids carriers are widely seen as the workhorses of drug delivery systems because of low toxicity, enhanced encapsulation capacity, controlled drug kinetic release, easy tailoring and targeting and practicable scale up. A new generation of hybrid lipid nanoparticles has emerged by combining the lipidic properties with polymers, proteins and metallic structures. The main features of hybrid lipid nanoparticles including popular methods for synthesis and characterization, biological and toxicological properties, administration routes, drug encapsulation strategies, tailoring and targeting, and potential systems for use in biomedicine are described in the present review.

Keywords: lipid drug delivery systems, lipid particulate systems, solid lipid nanoparticles, nanostructured lipid carriers, hybrid lipid nanoparticles, hydrophobic drugs, lipid toxicity

1. INTRODUCTION

Lipids are ubiquitous group of molecules and one of the basic constituent of the cells working as source of energy and signaling pathways as well as key components of cell membranes. Since the first works using lipids, researchers were trying to make and reproduce many types of cell-like membranes. Up today many lipid-based systems were developed such a vesicular model (*e.g*. archeosomes, liposomes, mesosomes, etc.) and lipid particulate systems (*e.g*. solid lipid micro- and nano-particles, nanostructured lipid carriers, etc.). In the last century, liposomes have widely and deeply investigated for biomedical and cosmeceutical applications [1,2]. The advantages and diversity of lipids motivates the develop a broad field in the pharmaceutical arena: lipid drug delivery systems (LDDS). Among the properties it can be mentioned the stabilization of drugs against harsh environments, increase the solubility of hydrophobic molecules, enhance permeability and adsorption of the drugs, and LDDS can be easily targeted. LDDS are very versatile for molecular delivery and used to develop ocular, oral, transdermal, pulmonary, parental administrations in the cosmetic and pharmaceutical industries.

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Particularly, liposomes have taken the lead for the development of LDDS. Amongst the major advantages of liposomes are easy to synthesize and scale up, variable chemical composition, diameters in the range of 50 to 500 nm, biocompatible and biodegradable, present low toxicity, able of stabilize therapeutic compounds and show moderate to fast uptake by the cells [3].

Considering these benefits, some encapsulated drugs in liposomes were approved and many are undergoing in pre-clinical and clinical trials for therapeutic use in acute pathologies of different types such as cancer, fungal infections and HIV [3,4]**.** However, liposomal technology suffers some drawbacks since are structures thermodynamically unstable (*i.e*. emulsions) changing the effective diameter with the time and consequently making the kinetic drug release more imprecise, which is a complex problem using very toxic drugs (*e.g*. anthracyclines). Also, the induction of complement activation and immunostimulation as well as toxicity were observed in repeated drug administration [4]. Different strategies were explored to circumvent the limitations of vesicular systems. One of the most promising systems developed during the early 90's were lipid particulate systems (*e.g*. liphospheres, solid lipid nanoparticles and nanostructured lipid carriers) [5,6]. Lipid particulate systems have the advantages of improving chemical stability of cargo molecules, water solubility, bioavailability and importantly structural stability. The fore front of lipid particulate systems are solid lipid

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nanoparticles (SLN) and nanostructured lipid carriers (NLC). Both type nanoparticulate lipid systems have the advantages of low acute and chronic toxicities, high tailoring capability using Green Chemistry techniques, good compatibility and feasible scale up, enhanced drug encapsulation and activity, controlled kinetic drug release following patient compliance [7,8]. SLNs are colloidal dispersion of lipids with high melting point in aqueous solutions (*i.e*. lipid matrix) in presence of surfactants and co-surfactants.

NLCs can be considered as second generation of lipid nanoparticle systems, which are improving drug loading by design the lipid matrix is less structured (*i.e*. low crystallinity) form by mixing solid and liquid lipids.

The main features, biological properties including potential toxicity, strategies of dug encapsulation and tailoring and targeting, hybridization with polymers and future perspective in biomedicine are reviewed in the present work.

2. LIPID NANOCARRIERS FEATURES

2.1. Physicochemical properties of Solid Lipid Nanoparticles (SLN), Nanostructured lipid carriers (NLC) and hybrid structures

SLNs and NLCs are based on lipid mixtures with specific molecular arrangement at nanometric scale, mainly because of surfactants present in the formulation, and whose purpose is to modulate the release of an active encapsulated molecule with specific biological activity. The lipid matrix of SLN usually is composed of solid lipids like triglycerides (caprylate triglyceride, trimyristin, tristearin), diglycerides (glyceryl palmitostearate, glyceryl dibehenate), monoglycerides (glyceryl behenate, glyceryl monostearate), fatty acids (myristic acid, stearic acid, palmitic acid) or waxes (like cetyl palmitate, miristyl miristate or carnauba wax) and they are employed in concentration ranging from 5 to 40% [9–11].

Physical stabilization of lipids in aqueous phase is produced by the addition of emulsifiers (surfactants) in the range of 0.5% to 5%, depending on the lipid composition. Among them, the most used are poloxamer 188 (Pluronic F68), polysorbate 80 (Tween 80), lecithin, tyloxapol, polyglycerol methylglucose distearate (TegoCare® 450), sodium cocoamphoacetate (Miranol® Ultra C32) or Solutol in addition to some natural and synthetic polymers like chitosan or PVA [12].

On the other side, NLCs are produced by adding liquid lipids to the lipid phase, like soy bean oil, oleic acid, capric triglycerides, squalene, isopropyl myristate or even essential oils [13]. The composition of a typical NLC is schematized in **Figure 1**. The mean size of lipid nanocarriers are in the range of 50-900 nm determined by DLS measurement or TEM observations [14].

Figure 1 Nanostructure lipid carriers (NLC). Scheme of drug loaded NLC (a); characterization of NLC particle size distribution by transmission electron microscopy (TEM) images (b). Figure obtained from **Islan et al., 2016** with permission [11].

Hybrid structures are obtained when the lipid matrices are combined with biocompatible polymers some of them reported in the literature such as poly (lactic-co-glycolic acid) (PLGA), dextran sulfate, chitosan, polyethylenimine (PEI) or polymers derived from soy bean oil [15,16].

The crystalline degree of lipid nanocarriers can be considered one of the most relevant physicochemical properties. Drug loading, stability during storage and release properties essentially rely upon the solid state of the particles [10]. In most of the preparation techniques of SLN and NLC, the lipids are heating above their melting point and hot microemulsions are produced. Despite they solidify after dispersion in the aqueous phase, not all lipid materials crystallize in colloidal states. In these cases, the lipid matrix may crystallize at temperatures much lower than their melting points, thus emulsions of supercooled melts are produced and the desirable nanoparticle dispersions are not obtained. To avoid this drawback, the hot colloidal emulsion should be cooled below the lipids critical crystallization temperature and stable emulsions of nanoparticles are formed. As an alternative, the nanoemulsions are cooled down to room temperature, while lipid droplets of the nanoemulsion re-crystallize to form the solid lipid nanoparticles. However, the SLN may coexist with other colloidal structures, such as micelles, liposomes, supercooled melts, and even drug nanoparticles [17].

In addition, crystallization of the lipid matrix could be retarded or suppressed if shorter chain monoacid triglycerides (tricaprin, trilaurin or trimyristin) or complex glyceride mixtures are used for SLN preparation. Besides, the use of longer chain triglycerides or emulsifiers with long saturated alkyl chains is highly recommended to obtain more stable formulations [18].

Liquid drugs or oils could also modify the crystallinity of the lipid nanoparticles, and transition from the first generation SLNs to improved NLCs is produced [19]. Depending on the disorder degree and the presence of imperfections that allows to accommodate the drugs in molecular form, three NLCs model are found. Type I or "imperfect crystal" are characterized by the presence of small amounts of oil drops producing highly disordered lipid matrix structure in which drug molecules and amorphous clusters of pharmaceuticals are located [20]. The type II or "multiple" type, is an oil-in-lipid-in-water system, where more amount of liquid lipids are incorporated to increase the solubility of lipophilic drugs, generating tiny oily nano-compartments within the solid lipid matrix [21]. The NLC type III or "amorphous" type, in which the solid lipid matrix is mixed with special lipids (*i.e*. hydroxyoctacosanyl hydroxystearate, isopropyl palmitate or medium chain triglycerides) to produce non-crystalline lipid nanoparticles [12].

For these reasons, two physicochemical techniques are very important to establish the crystalline degree and polymorphism of SLN, especially when novel formulations or preparation techniques are introduced: Differential Scanning Calorimetry (DSC) and X-Ray diffractometry (XRD) [10]. The DSC profile of samples allows to determine the melting point of the formulations and the crystallization

behavior of all components that are building the nanoparticles. Regarding to the lipid matrix, it could be observed shifts in the melting points and melting enthalpies of the lipids after the addition of oils or other molecules due to rearrangement of the crystalline structure [13,22]. On the other side, characteristic peaks of crystalline drugs can disappear if they are homogeneously distributed within the lipid matrix, remaining mainly in an amorphous state [11]. During the formation of hybrid structures the presence of the polymers could inhibit drug recrystalization, reason why peaks associated with the pure drug are not found suggesting an amorphous state of the cargo molecules [23].

By XRD analysis, the crystalline structure of different SLN components can be identified [10]. The XRD pattern becomes a useful tool to determine the presence of crystalline forms of the lipid molecules, changes in the phase behavior and rearrangements in the crystal structure due to the addition of oils, hybridization with polymers and the load of drugs [24]. XRD signal decrease of the peaks associated to the SLN after hybridization with protein structures (such as enzymes like DNase) can be observed, which suggests changes in the lipid spatial organization [11]. On the other side, the nanostructuration of SLN can be elucidated by disappearance or decrease of the peaks associated to the more stable lipid structure (B forms) and a concomitant increase in the peaks associated to the B´ metastable forms of the lipids, a desirable feature for drug encapsulation and storage [13].

2.2 Biological properties of SLN and NLC

Solid Lipid Nanoparticles (SLN), the first generation of lipid nanoparticles, emerged in the 1990s as an alternative drug carrier system combining the benefits of traditional systems employed (*i.e*. nanoemulsions, liposomes and polymeric nanoparticles) avoiding most of their drawbacks [6,25,26].

2.2.1 Advantages and disadvantages of lipidic nanoparticles with respect to other colloidal carrier systems

- SLN are composed by physiological biodegradable material such as triglycerides, fatty acids and waxes with GRAS status (generally recognized as safe, FDA, USA), most of them already in use in cosmetic and pharmaceutic industry. These features help to diminish the chronic and/or acute toxicity of SLN compared to some polymeric nanoparticles [27].
- SLN can protect loaded bioactive compounds against light and chemical or enzymatic degradation. Particularly, SLN are useful in oral delivery where the gastrointestinal tract offers harsh environmental conditions for drug delivery [28].
- SLN increase the bioavailability of lipophilic drugs, and at the same time, they can deliver both lipophilic and/or hydrophilic compounds [29].
- Unlike the synthesis of some polymeric nanoparticles, SLN synthesis does not involve the use of organic solvents and they can be easily scaled up to industrial level. High-yields, low costs, and very good reproducible SLN were obtained through the high-pressure homogenization methods [30,31].
- The characteristic solid matrix of SLN has several advantages over the liquid matrices present in other lipid nanocarriers [32]. Because of SLN not only protect loaded compounds in a better manner but also, avoid uncontrolled or burst release of cargo molecules, typically observed in nanoliposomes and nanoemulsions.
- SLN present better stability than liposomes which in general are thermodynamically unstable, high load efficiency than polymeric nanoparticles and formulations are stable at least for 3 years [33]. Moreover, lyophilization of SLN is possible.
- SLN have great potential to be administered via oral, topical, pulmonary and parenteral routes. Concerning oral route, besides protection to degradation by SLN in the gastrointestinal duct, they favor intestinal absorption. These features make them promising nanocarriers for orally administered drugs, considered the most effective methodology in terms of patient acceptance due to its non-invasive nature [34].

Despite of the several advantages previously highlighted, SLN present some limitations such as relative low loading capacity, which relies on drug solubility into the lipid matrix, variable percentage of water content and, probably the most relevant, drug release from SLN formulations during storage [35]. After synthesis of SLN containing high purity lipid matrix, a time-dependent restructuring process occur, leading to a low energy and perfect crystalline structure in the lipid matrix which minimizes imperfections and produces drug expulsion from the nanoparticle core [36].

Towards the end of 1990s and the beginning of the new century, the development of a second-generation lipid nanoparticles, called Nanostructured Lipid Carriers (NLC), appeared to overcoming some drawbacks associated with SLN [37]. The main different relies on the fact that NLC matrix is composed of a mixture of liquid and solid lipids instead of a unique pure solid and crystalline lipid. As a result, a less ordered solid matrix is obtained, which allows a greater load capacity and reduces drug expulsion during storage [38].

2.2.2 Considerations for *in vivo* and *in vitro* evaluation of SLN and NLC and their potential application through different administration routes

Several aspects are necessary to consider when considering SLN and NLC as potential drug nanocarriers, especially depending on the administration route intended. Among them, biocompatibility and biodegradability, sterilization, optimal pH and isotonicity of the formulation are the most required to establish [39,40].

Sometimes, SLN and NLC can be directly produced under sterile conditions. However, in most of the cases lipid nanocarriers need to be sterilized. For this purpose, autoclaving, filtration and gamma-ray exposition are feasible. The chosen method will depend on the nanoparticle structure to avoid alterations in the chemical and physical stability as well as in the drug release kinetic [41].

In the case of SLN and NLC containing heat-resistant drugs, autoclaving is the preferred choice. Besides, lipid

nanoparticles melt during the process, which could alter their physical stability, promoting aggregation or augmented size of nanoparticles [42]. The chemical nature of surfactants plays a critical role in autoclaving effect on SLN and NLC structure. Nonetheless, several authors reported a conserved stability of diverse SLN after autoclave sterilization [43,44]. Gamma irradiation is less frequently employed given that free radicals are produced promoting chemical modifications on the bioactive compound and the lipid nanoparticle itself [39]. Finally, filtration is feasible if the size of SLN and NLC does not exceed 200 nm [42]. Even though this last strategy was optimal in some situations, autoclaving is the most employed methodology given its low cost and simplicity.

On the other hand, despite the lack of consideration in most of the available bibliography, SLN and NLC formulations should be adjusted to physiological pH values before evaluation on *in vitro* and *in vivo* models [45,46]. In some cases, osmolarity must be adjusted to obtain isotonic formulations. To this purpose osmotically active substances can be added without forgetting that electrolytes like NaCl or KCl may destabilize lipid nanoparticle by altering their Z-potential [40].

2.2.3 Toxicity of SLN and NLC

Nanomaterial toxicity has gained great relevance in last year's due to the potential harmful effects based on the lack of biodegradability in most of the nanomaterials employed. As a result, increasing quantities of these materials accumulate in the environment and lastly, in the human body.

Several reports show that SLN and NLC are much less toxic than polymeric nanoparticles (10-100 folds at least) [47,48]. The great advantage of SLN and NLC over other colloidal carrier systems (*i.e.* metal and polymeric nanoparticles) relies on the relatively fast, easy and complete degradation, giving physiological products as a result including fatty acids and glycerol. Lipids and surfactants used in the carrier synthesis have almost, if not all, GRAS status and consequently, they are already approved for topical cosmetic and/or pharmaceutical purposes. However, SLN and NLC non-toxicity must be evaluated in depth, especially if they are intended to use in biomedical applications. Nanotoxicity should be ideally evaluated first *in vitro* (cultured cells), then *ex vivo* (tissue samples) and finally *in vivo* (animal models). Toxicity assays provide valuable information for preclinical studies about the biosafety of the formulations and the proper doses to be evaluated. Although *in vivo* studies in mice and rats provide most valuable information about toxicity in specific tissues as well as in the whole organism, there is a higher prevalence of *in vitro* studies reports regarding NLC and SLN toxicity, which is logical given that they allow to evaluate a wide spectrum of cell types and conditions in a more simple, economic and fast way.

A broad spectrum of methodologies is available to evaluate toxicology in cultured cells, most of them based on cell viability and proliferation assays that analyze mitochondrial activity (MTT, WST-1, resazurin), plasma membrane damage (LDH release, trypan blue, neutral red) and DNA synthesis (BrdU incorporation) [49]. MTT is, by far, the most employed assay to study lipid nanoparticles toxicity [50,51].

Nanoparticles could potentially trigger dangerous mechanisms without affect cell viability in a short term, leading to pro-inflammatory processes and cancer development. For this reason, some author also evaluates cytokine production, genotoxicity, oxidative stress and metabolic pathways involved in these pathologies [52].

Particularly, a reduction higher than 30% in cell viability is considered as cytotoxic effect [53]. *In-vitro* nanotoxicity could be evaluated using different cell models depending on potential application of the formulations (delivery of chemotherapeutic drugs, antibiotics, DNA, nutraceuticals) and routes of administration.

The IC50 value (concentration that inhibits 50% cell viability) is usually employed to compare cytotoxicity among different formulations. For SLN and NLC, most of the IC50 are found in the range of 0.1 mg/ml to 1.0 mg/ml [54,55] with some exceptions. SLN evaluated at concentrations as high as 2.5 mg/ml and 10 mg/ml resulted non-toxic in human dermal fibroblasts and human granulocytes, respectively [56,57]. The IC50 values obtained may result strongly dependent in the methodology selected, where MTT is commonly the most sensitive (lower IC50). Rocha et al. evaluated empty SLN and NLC in HaCaT cells using 4 different methodologies: MTT, Neutral red, Resazurin and Trypan Blue. The results showed that 0.1 mg/ml SLN and NLC significantly reduced cell viability evaluated by MTT (IC50= 0.3 and 0.7 mg/ml respectively), whereas no cytotoxic effects were found for the other 3 methods for concentrations up to 1.0 mg/ml [55]. Cell viability of NIH/3T3 and HaCaT cell lines cultured in presence of SLN and evaluated with MTT and neutral red showed similar results [58].

Exhaustive component analysis of SLN and NLC showed that surfactants are the major cause of cytotoxicity. Studies of peritoneal macrophages viability incubated with Dynasan®114-SLN formulations coated with different surfactants showed strong cytotoxic effect of the cationic surfactant cetylpyridinium chloride-coated SLN at concentration starting from 0.001% whereas no significant decrease was found for Lipoid-S75-coated SLN at concentrations up to 0.1% [30]. Similarly, tristearin-SLN containing SDS resulted more cytotoxic in Vero and MDCK cells than those bearing Tween 80 as surfactant [52]. Karn-orachai and coworkers analyzed the toxicity of SLN containing cationic, anionic or non-ionic surfactants in human skin fibroblast. They found that cationic surfactant SLN exhibited higher cytotoxicity in comparison to both other systems [59]. Cellular damage produced by surfactants is very high when they are in solution instead of immobilized onto SLN and NLC surfaces. This is a reasonable fact since immobilized molecules showed low interaction with cell membranes when they are attached to nanoparticle surface [60]. For this reason, lipid nanoparticle formulations should be designed to minimize the remaining free surfactant in solution by developing specific purification protocols such as ultrafiltration, ultracentrifugation or dialysis [61]. For SLN and NLC designed to intravenous (*i.v.*) application,

even most special attention is required given that surfactants, especially the ionic ones, may produce hemolysis [62]. The GRAS surfactants Poloxamer 188, sodium glycocholate, lecithin and Tween 80 are listed among surfactants accepted for *i.v*. application [63].

The concentration and nature of lipid matrices is the second most determinant factor in lipid nanocarriers toxicity. Even when most of the reports showed that lipid nanoparticles are non-toxic in the range of concentrations evaluated (usually up to 1.0 mg/ml), which use to be enough to reach therapeutic concentrations of loaded drugs, it was demonstrated that some neutral lipids reduce cellular viability at concentrations below 1.0 mg/ml. Schöler and colleagues found that SLN containing free stearic acid (0.01 mg/ml) were strongly cytotoxic in murine macrophages, and, in a lesser extent, those nanoparticles composed of triglycerides bearing stearic acid chains whereas SLN constituted by paraffin, other triglycerides or cetyl palmitate where well-tolerated [30]. In addition, stearic-acid-SLN showed high cytotoxicity in three different cell lines (J774A1 macrophages, 3T3 mouse BALB/c fibroblasts and HaCaT keratinocytes), and particularly in J774A1 cells [64]. Moreover, our research group analyzed the nanotoxicity of cetyl-palmitate, myristyl-myristate and cetyl-ester SLN over hepatocarcinoma (HepG2) and lung adenocarcinoma (A549) cells, resulting cetyl-palmitate-SLN toxic for both cell lines at 0.10 mg/ml and 0.15 mg/ml in HepG2 and A549 cells, respectively [13]. Similar findings were published elsewhere [65]. On the other hand, evidence suggest that differences in cytotoxicity is not dependent in SLN and NLC size nor cell phenotype evaluated (normal vs. tumoral) [30,66].

During the last years, reports evaluating *in vivo* toxicity of SLN and NLC notably increased, however, only a few works reported the cytotoxicity effects of empty nanoparticles. These reports are focused on the histopathological analysis of tissues which allow to support the biosafety of formulations tested. Brain-targeted empty SLN were tested in chicken embryos and rats showing not effect embryos development nor brain architecture [67]. In a more recent study, the cytotoxicity of SLN y NLC *i.p*. administered (200 mg/kg) in male Swiss albino mice for 2 weeks was analyzed. No significant differences in total weight, organs weight, hemolysis and biochemical parameters were found, concluding that lipid nanoparticles were well-tolerated [45]. Another work on systemic toxicity of aspirin and curcumin loaded-chitosan-SLN in BALB/c mice were reported. For that purpose, the mice survival, body weight, hematology, blood chemistry and organ histopathology after treatments were tested. No signs of acute, subacute or sub chronic toxicity (after 3, 28 and 90 days of treatment respectively) were found for empty or loaded-chitosan-SLN [68].

In conclusion, even when almost all components employed for SLN and NLC synthesis are considered with a GRAS status, and most of reports demonstrates biosafety of these lipid based nanoparticles, some exceptions were found depending essentially on the chemical nature and quantity of surfactants and lipid matrix utilized, therefore resulting essential to evaluate their potential toxicity on *in vitro* and *in vivo* models.

3. Drug encapsulation strategies on lipid carriers

The efficiency of drug encapsulation has been always a key feature to be optimized when lipid based nanocarriers are planned to be used for pharmaceutical purposes. Basically, drug incorporation depends on the structure and components of the carriers. Besides, each system has its own variations which depends on how drug molecules are located once inside the lipid system. Particularly, SLNs are divided in three different models in concordance with drug incorporation. Also, the nanocarrier structure is a consequence of formulation composition and production conditions [20]. First, Type I or homogenous matrix model which corresponds to a solid solution where the lipid and the drug are homogenously dispersed in their molecular state. In this case, a blend between the lipid and the drug is formed and the drug release is mainly governed by diffusion processes from the lipid matrix or by its degradation [17].

This structure is produced when SLNs are synthesized using cold homogenization methods. After homogenization, drug molecules are dispersed in the lipid bulk followed by vigorous agitation which leads to the formation of the nanoparticles. Recently, Metformin was incorporated into SLNs by cold homogenization method reaching 30% encapsulation efficiency [69]. Additionally, this type of structured nanoparticles was described when very lipophilic drugs are incorporated by hot homogenization technique [70]. Lipid droplets, formed after homogenization, are quickly cooled down avoiding separation between drug molecules and lipids. The method has been used many times for the incorporation of chemotherapeutic drugs. As an example, Paclitaxel, a highly lipophilic drug, showed promising results when encapsulated into SLNs by hot homogenization method [71].

The second model is named Drug-enriched shell and the drug is in the outer shell of the lipid carrier. This structure is formed when, during cooling step, the lipid crystallized before than the drug which generates a phase separation. Consequently, a drug free core, or with low drug amount, is formed. Meanwhile, the shell is enriched in the drug and precipitates comparable to the eutectic [20]. SLNs that suit this structure are characterized by a burst release of the drug. Some dermatological applications demand this kind of behavior for the administration of the active principle. For example, 5-Fluorouracil was incorporated into shell-enriched SLNs with the purpose of delivering a highly penetrating carrier that release high drug doses for skin carcinoma treatment [72].

The third type of SLNs structure after drug incorporation corresponds in a certain way to the opposite of Type II. It was named “drug enriched core model” and occurs when drug molecules precipitate before the lipid. When drug is well-dissolved in the lipid phase or when it is near the saturation limit, a cooling step during synthesis allow the formation of a super-saturated solution in small volume and consequently the drug crystallizes before lipid matrix [72].

As the new second-generation carriers, NLCs were developed to minimize SLNs drawbacks. In the same way, three morphologies were described for NLCs related to drug incorporation and location into the lipid matrix. Mixing solid lipids with a small quantity of a liquid lipid during NLCs

synthesis will give place for the formation of Type I structure or "imperfect crystal". In this case the solid matrix structure has imperfections were drug molecules can be fit. Maximizing imperfections generates more space for drug molecules, enhancing encapsulation efficiency. Using small amounts of liquid lipids generate the imperfections on carrier structure [73]. Besides, interaction between liquid lipid (oil) and the drug is very important to achieve high encapsulation efficiencies. Typical example is the encapsulation of lansoprazole, a drug used to prevent acid production in the stomach, in NLC. The absence of oil phase in the NLC formulation significantly reduces the incorporation of the drug in the nanoparticles [74]. NLCs formed by special lipids that avoid crystallization are named "amorphous type" or Type II. In this type of structure, the solid lipids are mixed with some special liquid lipids like isopropyl palmitate. The carrier solidified in amorphous state (do not crystallize). Therefore, nanoparticle core remain in an amorphous phase and, consequently, drug expulsion of the matrix is minimized [70]. Finally, NLCs Type III or Multiple Type are obtained by oil-in-fat-in-water dispersion. In this sense, small nano-compartments of liquid oil are formed inside the solid structure of the carrier by a separation of phases [12,75].

Hybrid lipid nanocarriers like lipid-polymeric nanoparticles (LPNs) are recently getting attention in research because of their advantages in drug encapsulation and rational design. The physicochemical and biological properties of matrix components, e.g. polymers and lipids, as well as the drugs were taken into the account for the design of the nanocarrier. One of the most important parameters for modulating encapsulation efficiency is lipid/polymer mass ratio (L/P ratio). In this regard, the lipid coating acts as a barrier that keeps the encapsulated drug inside the polymer core, resulting in high encapsulation efficiency [76]. In addition, LPNs structure allow to encapsulate either hydrophilic in lipophilic active principles, or even both. Recently, the development of LPNs loaded with doxorubicin (DOX) and indocyanine green for combined chemo-photothermal therapy was reported [77].

4. Lipid nanocarrier surface modification for enhance therapeutic efficacy: active targeting

One of the major aims of drug delivery research is to obtain systems with high therapeutic index, which is the ratio between drug therapeutic effect and side effects. When the drug has low therapeutic index, it has to be delivered in a higher concentration to target cells [78]. Active targeting consists basically on this approach, having as a main objective the generation of more effective therapies. In this regard, lipid nanocarriers have been extensively surface modified to achieve active targeting properties. For example, lipid carriers are targeted to specifically interact with receptors located on cancer cell membrane in cancer therapy. In this way, the anti-cancer active principle is delivered predominantly to cancer cells and therapeutic side effects could be reduced [79]. Many different structures can be attached to lipid nanoparticles to target cancer cells. During the last years carbohydrate molecules have gained substantial attention in this sense. Neoplastic cells express several membrane receptors, named lectins, that have a particular empathy for some carbohydrates like galactose, mannose, fucose, fructose and lactose [80]. Recently, galactosylation of SLNs was performed to improve the delivery of Doxorubicin to lung cancer cells. Promising results showed high affinity of modified SLNs to A549 lung cancer cell line in comparison with the carrier without modification [81]. In another study, Garg et al. decorated SLNs with fucose for breast cancer treatment. The study demonstrated that targeting SLNs loaded with Methotrexate have greater cellular uptake and better cytotoxicity. Additionally, *in-vivo* evaluation showed tumor targeting efficiency with minimum secondary effects when compared with free drug. Besides, fucose modified SLNs exhibited higher drug accumulation in tumor than unmodified nanocarrier [82].

Another ligand that has been used for cancer targeting is Transferrin (Tf). Cancer cells usually over express Tf receptors (TfR) makings them effective targets for active targeting and site-specific delivery of therapeutics. The interaction Tf-TfR helps in the uptake of iron and the Tf binding is followed by endocytosis [83]. An interesting NLC system was presented by Han and co-workers where they co-encapsulated Doxorubicin and enhanced green fluorescence protein plasmid (pEGFP) for drug and gene delivery. The nanocarrier was surface modified with Tf ligands for targeting lung cancer. The results showed that Tf-NLCs can improve the gene transfection efficiency and control the tumor growth rate on tumor-bearing mice compared with non-modified counterparts [84]. In another work, NLCs surface was modify using Tf and hyaluronic acid (HA) ligands for gene delivery into lung cancer cells, as well. The results showed that NLCs decorated with both ligands increase transfection efficiency than undecorated and single ligand-decorated NLCs *in vitro* and *in vivo* [85]. HA is a glycol amino glycan with an important role regulating different cellular responses by binding to cell specific receptors such as glycoprotein CD44. It has already been reported that the CD44 receptors are highly expressed in majority of non-small cell lung cancers (NSCLC) [86]. Moreover, HA decorated NLCs, loaded with paclitaxel, were also assayed against a melanoma *in vivo* model. The results showed a better tolerability and an increased in antitumor activity in B16-bearing Kunming mice compared with Taxol. Additionally, it was found that the lipid carrier could extend the circulation time of paclitaxel in the blood and increase the accumulation in the tumor [87].

In a different approach, certain modifications allow to enhance the mucus-penetrating properties of orally administered nanocarrier. Hydrophilicity and neutral charge are the desired properties towards mucus-penetrating nanoparticles [88]. In this sense, Beloqui and co-workers reported an interesting ligand that, once attached to NLCs, could provide mucophilic properties. Dextran and protamine were linked together to form complexes (Dex-Prot), then a coating on NLCs was obtained. Dextran provided a shield against NLCs-mucins interactions and Protamine enhanced cell-uptake as a cell penetrating peptide. Results demonstrated that Dex-Prot coating enhances the permeability of a lipophilic drug across an *in vitro* mucus model [89]. Moreover, other polymers such as polyethylene glycol 400 (PEG400), polyvinyl alcohol (PVA) and chitosan

(CS) have been studied for providing mucoadhesive properties. A very complete study confirmed that polymer-coated NLCs showed greater physical stability and mucoadhesion properties as compared to uncoated NLCs. Particularly, PEG and PVA exhibited strong interaction with porcine intestinal mucosa. This property is useful to prolong the contact time of the drug delivery system in GI tract [90]. Additionally, CS decorated NLCs were used to deliver active principles by intranasal administration with the ability to cross the blood-brain-barrier (BBB). Glial cell-derived neurotrophic factor (GDNF) is a potent neurotrophic factor that acts in the promotion of the survival and differentiation of dopaminergic neurons. GDNF was incorporated into the modified NLCs with the aim to treat neurodegenerative diseases like Parkinson. The results suggested that an improvement in the nose-to-brain delivery of the neurotrophic factor after intranasal administration. This results were adjudicated to CS good mucoadhesive properties, together with penetration enhancement properties across mucus epithelia [91].

Among the target molecules for oral delivery, Biotin has been also studied. Biotin, also known as Vitamin H or coenzyme R, is involved in many physiological processes and it is absorbed by receptor-mediated endocytosis [92]. Biotin decorated NLCs were used to encapsulate the natural compound oridonin (Ori) for oral delivery. Biotin coating demonstrated to enhance oral bioavailability of the encapsulated active compound [93].

LPNs have been decorated with several surface modifications for targeting purposes, as well. In this case, the ligand molecules could be the same that in full-lipid carriers but the modification strategy needs to be adapted **(Table I)**.

Table I. LPNs with active targeting moieties

Targeting molecules has to be conjugated to the lipid or lipid-PEG precursors prior to the LPNs synthesis or conjugated to the lipid shell of the LPNs post-LPNs preparation [76]. LPNs loaded with docetaxel were decorated with the A10 RNA aptamer to specifically target prostate cancer cells overexpressing the prostate specific membrane antigen [94]. Folic acid (FA) is another traditional targeting moiety, can be easily recognized by folate receptors over expressed in many cancer cells. LPNs were prepared with PLGA and 1,2-distearoyl-snglycero-3-phosphoethanolamine (DSPE)-PEG-COOH and DSPE-PEG-FA. Results indicated that the FA-targeted LPNs exhibited better uptake and higher cytotoxicity in MCF-7 cells compared with non-targeted carrier [95]. In another interesting work, the tumor-targeting peptide iRGD was used to decorated LNPs for co-delivery of doxorubicin and sorafenib to enhance anti-hepatocellular carcinoma (HCC) efficacy [96]. Through the iRGD-integrin recognition, these LNPs showed synergistic cytotoxicity, pro-apoptotic ability and enhanced internalization rate in human liver cancer HepG2 cells. Besides, iRGD-LPNs enhanced antitumor efficiency in HCC xenograft mouse models.

5. Preparation methods of hybrid lipid nanoparticles

Up today, five techniques were reported to synthesize lipid nanocarriers such as hot microemulsification - ultrasonication, solvent diffusion, spray-drying, high-pressure homogenization and microemulsification combined with solvent evaporation [97]. Despite of several techniques used to produce SLN and NLC, the following two main procedures are the most reported to obtain hybrid lipid nanostructures and the schemes are displayed in **Figure 2** [76].

5.1 Microemulsification - ultrasonication

The procedure mainly involves dispersing techniques of warm emulsions [11,24,98,99]. In a first step, the solid lipid (with the addition of oils or the drugs) is heating to approximately 5–10°C above its melting point. Then, the lipid mixture forms an emulsion when is dispersed in an aqueous surfactant solution containing the polymers (carbohydrates, proteins or synthetic polymers) at the same temperature under high speed stirring. The resulting emulsion is exposed to ultra-sonication to reduce the droplet size. Finally, the microemulsion is gradually cooling down below the crystallization temperature of the lipid to produce lipid nanoparticle dispersion with homogenous size distribution (**Figure 2a**).

5.2 Emulsification-solvent diffusion

In this technique, the lipids and the drug are solubilized in partially water-miscible solvents (such as benzyl alcohol, butyl lactate, isobutyric acid, isovaleric acid and tetrahydrofuran) that are preliminary saturated with water to ensure a thermodynamic equilibrium between the liquids (internal phase). Then, this organic phase is emulsified with a solvent-saturated aqueous solution containing an emulsifier and polymer (dispersion medium) using high shear homogenizer. Finally, water is added to the primary emulsion to extract the solvent into the external water phase, precipitating the lipid and thus forming the SLNs (**Figure 2b**) [15,100,101].

Figure 2 Scheme of the most used methods for preparation of one type of hybrid lipid nanoparticle: a) emulsification/ultrasonication; b) solvent diffusion method.

6. Development of new hybrid SLN/NLC models

The development of novel hybrid lipid nanoparticle designs open a new alternatives for the delivery of different kind of molecules and particularly those with hydrophilic properties [99,102]. In addition to NLC improvements to effectively incorporate lipophilic mixtures, a new generation of nanoparticles has emerged to increase the ability of nanocarriers to transport hydrophilic drugs, ionic mixtures and numerous biocompatible and biodegradable lipids [76]. New nanoparticle designs facilitate the receptor-mediated membrane adhesion [103]. Among them, polymer-lipid hybrid nanoparticles and lipid-drug conjugate nanoparticles appear as innovative alternatives for molecular controlled release [63].

Considering the chemical nature of the non-lipid components, three hybrid structures can be described: lipid-polymer, lipid-protein and lipid-metallic.

The lipid-polymer hybrids become an alternative for the transport of hydrophilic drugs that are commonly used in salt forms for their clinical application. Since the presence of ionic charges may lead to low drug incorporation into lipid nanoparticles, the formation of drug-polymer complexes with a compatible design with the hydrophobic matrix is a good choice to overcome this drawback [76]. In addition, these systems are interesting for their ability to delivery multiple drugs [98]. Based on the lipid-polymer ratio two lipid-polymer models can be found (**Figure 3**).

Figure 3 Models for lipid-polymer hybrid nanoparticles: a) polymer core and lipid shell; b) lipid core and polymer coating. Drugs accommodate within the matrix according to the molecular nature.

Hybrid structures can provide very interesting enhanced therapeutic properties. For example, Dox-loaded hybrid solid lipid nanoparticles showed over 8-fold increase in cell dead of tumoral cells (MDR line) in comparison with free Dox at equivalent doses. Those nanoparticles were obtained when cationic Dox was complexed with soybean-oil-based anionic polymer and both were dispersed with a lipid in an aqueous phase [102]. The efficacy of the nanoparticles was tested in animal models showing high cytotoxic activity *in vivo* against solid tumors and minimal systemic toxicity [99]. In another approach, the importance of the interactions between drug-polymer-lipid for the rational design of hybrid lipid nanoparticles was studied. The use of anionic polymers (polyelectrolyte, such as dextran sulfate sodium) becomes an interesting tool to enhance drug loading and increase cytotoxicity against MDR cancer cells [24,104]. In a similar study, Dox and mitomycin C were co-encapsulated in polymer-lipid hybrid nanoparticles, finding an increase of cancer cells apoptosis in a orthotopic murine breast tumor model [16]. In addition, those nanoparticles released DOX in tumors more efficiently than the liposomal formulation. Also, a synergism between both encapsulated drugs was observed for treating lung metastases of triple negative breast cancer in mice [105]. In previous report, decorated polymer–lipid hybrid matrix (PLGA–lecithin–PEG core–shell nanoparticles) with folic acid showed interesting *in vitro* and *in vivo* results for glioma targeting in brain delivery of a modified version of paclitaxel [106]. Hybrid hyaluronic-lipid nanoparticles demonstrated antitumor efficacy against hepatocellular carcinoma [85–87] Also, the preparation of ultra-small nanoparticles becomes an interesting design to increase the tumor penetrating ability of drugs [107].

In other applications, surface modified polymer–lipid hybrid nanoparticles were developed by combining hydroxypropyl methylcellulose with lipid phase (dynasan and stearylamine) for intranasal delivery of ropinirole in treatment of Parkinson's disease [100]. An interesting carrier was prepared by combining the advantages of gelatin (cationic polymer) and lecithin (anionic lipid) to produce gelatin coated hybrid lipid nanoparticles that increase the oral bioavailability of Amphotericin B [108]. Also, hybrid solid lipid nanoparticles with hyaluronic acid were proposed as a novel tool for gene delivery [109].

Among the lipid-protein hybrid nanoparticles, the use of enzymes with biological activities absorbed on SLN surface could enhance the therapeutic properties of encapsulated drugs and generate synergisms effects [11]. In addition, cationic lipid nanocarriers can adsorb peptide hormones (like insulin) onto their surface, increasing the bioadhesion properties and bioavailability [110]. Surface modification with peptide ligands led to an increase on oral bioavailability of drugs and particularly, improving the transport across intestinal barriers [111].

Finally, hybrid lipid-metallic nanoparticles were already developed as pharmaceutical nanocarriers to change the pharmacokinetic and biodistribution of encapsulated drugs [112]. Nanoparticles made of iron oxide cores embedded in a solid lipid matrix (glyceryl trimyristate) showed promising *in vitro* results against human HT29 colon adenocarcinoma cells after hyperthermia treatment [113]. On the other side, hybrid lipid-metal nanoparticles become an interesting tool for the development of nano-antibiotics. A silver complex of clotrimazole was effectively encapsulated into solid lipid nanoparticles and exhibited a significant antibacterial activity against *S. aureus* and methicillin-resistant *S. aureus* [114].

7. Potential uses of lipidic nanocarriers in biomedicine and future perspectives

Several reviews of lipidic nanocarriers have been published in the last years. These reviews basically discuss the types of nanostructured lipid carriers (NLCs), mechanism of skin penetration, stability related issues along with their production techniques, characterization and applications towards targeted drug delivery [19,34,115–117]. The raise growth of the NLC exploitation was essentially due to defeated barriers within the technological process of lipid-based nanoparticles formulation and increased knowledge of the underlying mechanisms of transport of NLC via different routes of administration. This overview on novel NLC applications providing examples of successful outcomes [118].

NLCs modified by polyethylene glycol (PEG) (PEG-NLC) loaded with, hydroxycamptothecin (HCPT) were prepared by high pressure homogenization and spray drying method. PEG-NLC was characterized by scanning electron microscopic (SEM), powder X-ray diffraction (XRD) and differential scanning calorimetry (DSC). HCPT was released from PEG-NLC in a sustained profile. After storage for 6 months, PEG-NLC powder after spray drying showed no changes in term of particle size, drug loading and entrapment efficiency, crystal form and release. The phagocytosis of pegylated NLC on RAW 264.7 cells, decrease significantly the cellular uptake of PEG-NLC compared with NLC. The authors suggested that PEG-NLC formulation prepared by this method was stable and resulted in a high-performance delivery system for HCPT [119].

Celecoxib-NLC (CXB-NCL) was formulated for the treatment of inflammation and allied conditions. CXB-NLC was prepared by microemulsion template technique (Lipid phase (1) Solid lipid: Glyceryl dilaurate. (2) Oil phase: Capmul MCM. Surfactant phase (3) Surfactant phase: Cremophor RH 40. (4) Solubilizer: Transcutol. (5) Aqueous phase: double distilled water). The nanoparticulate dispersion was gelled and tested for *in vitro* release and *in vitro* skin permeation in rats. Efficacy of the CXB-NLC gel was established using aerosil-induced rat paw edema model. The CXB-NLC based gel showed faster onset and elicited prolonged activity until 24 h [120].

Nifedipine (NI) is a poorly water-soluble drug and its oral bioavailability is very low. To improve the water solubility, NI-lipid nanoparticle suspensions were prepared by a combination of co-grinding by a roll mill and high-pressure homogenization in presence of hydrogenated soybean phosphatidylcholine (HSPC) and dipalmitoyl phosphatidylglycerol (DPPG) and without any organic solvent. NI-NCL remained stable during a period of 4 months under dark conditions at 6ºC, suggesting that the negative charge of the phospholipid, dipalmitoyl phosphatidylglycerol, is very effective for preventing coagulation of the particles. Freeze-drying in the presence of sugars (glucose, fructose, maltose or sucrose) added to the suspensions before freeze-drying inhibited the aggregation of nanoparticles, and a increased the solubility [121].

A NCL constituted by thiolated non-ionic surfactant, cysteine-polyethylene glycol stearate (Cys-PEG-SA) was synthesized in two steps reaction involving a new derivative intermediate formation of p-nitrophenylcarbonyl-PEG-SA (pNP-PEG-SA). The nanostructured lipid carrier (NLC) loaded cyclosporine A (CyA) was prepared by melt-emulsification method (lipid matrix consisted of Precifac® ATO 5 and Miglyol® 840) and CyA relative to total lipid was mixed and melted at 80°C, then dispersed in aqueous solution of Tween 80 and polyethylene glycol 40 monostearate (PEG-SA) stirred to form a coarse emulsion, followed by sonication and cooled at 0°C in order to solidify the lipid matrix and form NLC-CyA. The mucoadhesive Cys-NLC-CyA was prepared by incubating NLC-CyA emulsion with Cys-PEG-SA. The mucine particle method using porcine mucin particles was used for the measurement of mucoadhesive properties. Cys-NLC-CyA administered topically to the rabbit eye showed that the encapsulated cyclosporine remained on the ocular surface in the cul-desac for up to 6 h. Either pre-corneal retention time and concentration was highly increased, as compared with the NLC without thiomer modification [122].

NLCs prepared from amixtures of Precirol (solid lipid nanoparticles, SLNs) and squalene (lipid emulsions, LEs) loading lovastine were prepared to investigate the bioavailability of lovastatin by oral delivery. Higher lovastatin loading in NLCs and LEs was afforded compared to SLNs. The *in vitro* release kinetics demonstrated that lovastatin release could be reduced significantly with lipid nanoparticles containing Myverol as the lipophilic emulsifier. The *in vivo* studies indicated superior stability of the Myverol system over the soybean phosphatidylcholine system in the gastric environment [123].

Celecoxib (CXB) in the NLC (prepared with CXB, compritol, miglyol and sodium taurocholate using high pressure homogenization) were applied for nebulization of Balb/c mice. *In vitro* cytotoxicity studies showed dose and time dependent cytotoxicity against A549 cells. The lung deposition and pharmacokinetic parameters of CXB-NLC and Cxb solution (CXB-Soln) formulations were determined and nebulization of CXB-NLC demonstrated higher AUCt/D in lung tissues and lower systemic clearance as compared to the Cxb-Soln. Cxb encapsulated NLC exhibited stability and aerodynamic properties within the respirable limits. Then, aerosolization of CXB-NLC improved the CXB pulmonary bioavailability compared to solution formulation [124].

Dihydro-artemisinin (anti-malarial) nanostructured lipid carrier (DHA–NLC) were prepared by solvent diffusion method using glycerol monostearate (MS), mygliol-812 and Tween-80. The *in vitro* experiments proved that DHA-NCL released gradually over the period of 48 h, exhibiting a biphasic pattern with burst release at the initial stage and sustained release subsequently [125].

Antitumoral bufadienolides-loaded NCLs (BU-NLC) were prepared for parenteral application using glyceryl monostearate, medium-chain triglyceride, oleic acid, and Lipoid E-80®, sodium deoxycholate and pluronic F68 as stabilizers. BU-NLC cytotoxicity was *in vitro* evaluated on human astrocytoma cell line (U87-MG) and human gastric carcinoma cell line (HGC-27), showing similar antiproliferative effects in comparison to the free drug. The pharmacokinetic study demonstrated an improved profile, resulting in a higher plasma concentration and lower clearance after intravenous administration as BU solution (BU-S). A sarcoma-180 tumor model further confirmed the advantages of BU-NLC versus BU-S. Acute toxicity and hemolysis studies showed that BU-NLC was safe when given by intravenous injection [126].

A lipophilic cardiovascular drug, Tanshinone IIA-loaded HDL-like nanostructured lipid carriers (TA-NLC) were prepared by a nanoprecipitation/solvent diffusion method. Phagocytosis and cytotoxicity was evaluated using mouse macrophage cell line RAW 264.7 with TA-NLC and incubated TA-NLC with native HDL (TA-NLC-apo). The incorporation of glycerol trioleate to NLC led to a crystal order disturbance. SDS-PAGE patterns indicated that TA-NLC could bind to apolipoprotein A-I (apoA-I) specifically *in vitro*. Phagocytosis studies showed significant differences in uptake between TA-NLC and TA-NLC-apo and demonstrated that TA-NLC incubated with native HDL could turn endogenous by association to apolipoproteins, which could not trigger immunological responses and escaped from recognition by macrophages [127].

A NCL composed of precirol, myverol, and pluronic were able to form stable dispersions with a high encapsulation of the lipophilic drug lutein (phylloquinone). The lutein-loaded NLCs had a sustained release of the drug in simulated intestinal fluid compared to simulated gastric fluid using modified Franz diffusion cells [128].

Date et al. (2011) prepare repaglinide NLCs with Gelucire 50/13 as an amphiphilic lipid excipient in the presence of Precirol® as anti-diabetic drug. Gelucire 50/13(stearoyl macrogolglycerides) has been previously used for the

preparation of solid dispersions for improving the aqueous solubility of lipophilic drugs. Through DSC studies it was found that Gelucire 50/13 interacts with Precirol® suppressing polymorphic transitions of both components. The NLCs exhibited a greater decrease of the blood glucose level in rats compared to repaglinide commercial tablets [129].

In bromocriptine loaded NLCs the *in vivo* results showed that bromocriptine NLCs had a rapid onset of action, longer duration and higher brain levels as compared to that of solution. Moreover, the entrapment efficiency was also increased [130].

Tacrolimus loaded NLCs (TAC-NLC) that it was prepared by glycerol monostearate and dimethylglycol monoethyl ether by the hot sonication and homogenization technique. TAC-NCL exhibited penetration rate through the skin of a hairless mouse greater than that of Prototopic®. *In vitro* penetration tests revealed that the tacrolimus-loaded NLCs have a penetration rate that is 1.64 times that of the commercial tacrolimus ointment, Protopic® (TAC commercial drug) [131].

Triamcinolone acetonide (TA) is a corticosteroid drug currently administered by intravitreal injection for a broad spectrum of inflammatory, edematous and angiogenic ocular diseases. To increase the drug's bioavailability by ocular instillation, TA was encapsulated in nanostructured lipid carriers (TA-NLC), (composed of Precirol®ATO5 (solid lipid), a mixture of mono-, di- and triglycerides of palmitic acid (C16) and stearic acid (C18), Lutrol®F68 (hydrophilic surfactant), a hydrophilic block copolymer of ethylene oxide and propylene oxide, Squalene® (liquid lipid), rac-1-oleoylglycerol (lipophilic surfactant)). Based on the selected formulations, *in vivo* tests were carried out by eye-drop instillation of TA-NLC in mice, revealing the systems' ability of delivering lipophilic actives to the posterior segment of the eye via the corneal and non-corneal pathways. This TA-NLC showed during a period of 6 months, a low tendency of these particles for aggregation during shelf life when stored at room temperature [132].

An important pH-sensitive membranolytic and lysosomolytic nanocarriers (BSA-AL-NLCs) were prepared by loading l-arginine lauryl ester (AL) into NLCs and coating with bovine serum albumin (BSA). Hemolysis assay demonstrated that BSA-AL-NLCs did not disrupt biomembrane at pH 7.4 and presented ideal feasibility as lysosomolytic nanoparticles without cytotoxicity. However, hemolysis assay demonstrated the pH-sensitive biomembrane disruptional capability of AL. Lysosomolytic capability of BSA-AL-NLCs was shown after internalized into MCF-7 (human breast cancer cell) via endosome–lysosome pathway. Loaded BSA-AL-NLCs-Paclitaxel (PTX) displayed pH-dependent release *in vitro* and no cellular toxicity was found on MCF-7. The anti-tumor effect of PTX-loaded BSA-AL-NLCs was more efficient than to BSA-NLCs, indicating that AL served to facilitate lysosomal escape of BSA-ALNLCs improving the anti-cancer effect. *In vivo* experiments showed that bio-distribution and anti-cancer activity confirmed the improved tumor targeting and anti-cancer efficacy of BSA-AL-NLCs. The authors suggested that a small molecule, such as AL may afford more nanocarriers lysosomolytic capability with lower cytotoxicity, with an improvement the therapeutic index of loaded active agents [133].

Monoolein aqueous dispersions (MADs) and bromocriptine-NLCs (BC-NCL) (ultrasonication, lipid mixture was constituted of tristearin/Miglyol, aqueous poloxamer 188 and BC) were compared as antiparkinsonian drug BC delivery systems. In this case both dispersions and BC-NLCs. BC-NLCs provided prolonged therapeutic effects of BC *in vivo* in a 6-hydroxydopamine lesion (6-OHDA), a Parkinson model in rats (male Sprague-Dawley rats) following an intraperitoneal administration [134].

Curcumin-loaded NLCs (CUR-NLC) (prepared by ultrasonication with Precirol ATO 5, Miglyol 812, Lutrol F68 and Tween-80 and CUR) were tested in CD1 mice and reported that CUR-NLCs could decrease histone acetylation in the central nervous system (CNS) after intraperitoneal injection. The authors were suggested a novel approach to ameliorate the pharmacokinetics of CUR that allows a better permeation in the CNS [135].

An intravenous delivery system of β-Elemene (β-E) in NLC was prepared using high pressure homogenization with glycerol monostearate as the solid lipid and a mixture of Maisine 35-1 and Labrafil M1944 CS as the liquid lipid. β-E-NLCs were less irritating, less toxic and showed significantly higher bioavailability and anti-tumor efficacy than the free β-E injection [136].

Using budesonide-loaded NLCs (BDS-NLCs) (prepared by using the high-pressure homogenization technique with Precirol ATO®5, Miglyol 812, Tween 80 and poloxamer 188) for reducing inflammation in colitis and it was found that after 24 h, both loaded and unloaded NLC formulations significantly reduced tumor necrosis factor (TNF)-α secretion when compared to untreated LPS-activated macrophages. The evaluation in a murine dextran-sulfate (DSS)-induced colitis *in vivo* model found that 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. No differences were observed from the healthy group when mice were treated with either unloaded-NLCs or BDS in suspension. NLCs were observed after 12 h in the colon of DSS-treated mice after oral administration. The authors suggested that all these results justify the use of NLCs for anti-inflammatory drug delivery in the gastrointestinal tract. This open to new research on the anti-inflammatory of NCL in inflammatory bowel disease treatment [137].

The mechanisms of transport of curcumin (CUR)-loaded NLCs across human and mouse lung microvascular endothelial cells (LMVECs) isolated from wild-type (wt) and Caveolin-1 knockout (Cav-$1^{-/-}$) mice was reported. Since the absence of Caveolin-1 did not lead to an uptake of CUR-NLCs, they need caveolar vesicles for their internalization. Immortalized Human Pulmonary Microvascular Endothelial cells (HPMEC-ST1) monolayers were challenged with thrombin to induce endothelial permeability and the presence of CUR-NLCs preserved this effect in a dose-dependent manner and prevented actin cytoskeletal reorganization and intercellular gap formation.

The same results were found *in vivo* (*e.g.* acid aspiration murine acute lung injury model (ALI)). The authors concluded that CUR-NLCs preserve lung microstructure in ALI [138].

A multifunctional NLC-containing anticancer drugs Dox or paclitaxel (TAX)), siRNA targeted to *MRP1* mRNA as a drug resistance suppressor, siRNA targeted to *BCL2* mRNA and an analog of luteinizing hormone-releasing hormone (LHRH) (a ligand for lung cancer cells' membrane receptors) was reported. LHRH-TAX-NLC-siRNA significantly decreased the expression of both *BCL2* and *MRP1* when compared to untreated or free TAX treated cells. *In vivo* study (orthotopic lung cancer model in athymic *nu/nu* mice) revealed that 83% of non-targeted NLC-TAX-siRNA was retained in the lungs, around 4-fold higher accumulation when compared to intravenously administered NLCs. LHRH-NLC-TAX-siRNA-treated mice exhibited the lowest tumor size at day 24 and half of the animals exhibited levels of cancer cells below the limit of detection, showing total elimination of the lung tumor in the experimental disease [139].

Loading of Zerumbone (ZER) into NLC (ZER in lipid melt was dispersed in the aqueous surfactant solution of Sorbitol, Tween-80, and thimerosal in water) will increase the bioavailability of the insoluble ZER in the treatment of cancers. The formulation showed sustained-release characteristics and had high cytotoxicity in a human T-cell acute lymphocytic leukemia cell line (Jurkat T-cell line J.RT3-T3.5). Both ZER and ZER-NLC exerted their antitumor activity via caspase-9 and capase-3 activation, inhibiting anti-apoptotic proteins and stimulating pro-apoptotic protein expression. The authors suggested that ZER-NLC formulation has potential as a new delivery system for ZER in the treatment of leukemia [140].

Transferrin (Tf)-containing ligands were coated onto the surface of NLCs (lipid dispersion was composed of Precirol ATO-5, olive oil, lipoid S100, soybean lecithin and DOX, while the aqueous phase was prepared by dissolving pEGFP-N1(fluorescente probe), Tween-80, and dimethyldioctadecylammonium bromide (DDAB) in 10 mL of water) as targeting moieties. *In vitro*, acting on A549 cells, Tf-NLCs exhibited higher transfection efficiency when compared to non-targeted NLCs. *In vivo* (male C57BL/6 mice), Tf-NLCs showed better antitumor efficiency when compared to free DOX in terms of tumor growth [84].

Beloqui et al. (2014) studied the effect of the intestinal mucus layer on saquinavir (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 [141,142]. SQV permeability values were significantly lower across Caco-2/HT29-MTX cell monolayers when compared to Caco-2 cell monolayers for all tested formulations, indicating that the mucus layer hinders NLC access to the underlying epithelium and represents a barrier to overcome for NLC formulations. It was also found also that the coating of NLCs with a dextran–protamine complex (Dex–Prot) significantly increased 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.

In vitro (dialysis membrane diffusion technique) and *in vivo* (male Wister albino rats) studies of anionic (shear homogenization, Compritol 888 ATO and Labrafac™ Lipophile WL1349; surfactants: P188: Tween 80, deoxycholic acid sodium salt) and cationic (same as before except adding stearylamine) nanostructured lipid carriers (NLCs) loaded with ropinirole (RP) (RP-NLC) were carried out. The anionic RP-NCLs showed mild to moderate reversible inflammation of the nasal epithelium in rats, and destruction of the lining mucosal nasal epithelium with the cationic RP-NLCs. The absolute bioavailability of both drugs was enhanced compared to that of the intranasal solution (IN). Cationic RP-NLCs *in situ* gel showed a non-significant higher Cmax (maximum concentration) in the brain compared to the anionic RP-NLCs. Anionic NLCs in situ gel gave highest drug targeting efficiency in the brain (DTE) than of the cationic NLCs [143].

Devkar et al. (2014) developed Ondansetron (OND)-loaded mucoadhesive NLCs (OND-NCL) (high pressure homogenization, glycerol monostearate [solid lipid] and Capryol 90 [liquid lipid]. Soya lecithin [SL] and poloxomer 188 were chosen as surfactant and co-surfactant, respectively). OND-NLCs were tested *in vivo* (male Sprague Dawley rat) for intranasal delivery to the brain and observed that the intranasal route helped to achieve a higher drug targeting and direct transport to the brain compared to intra venous administration of plain drug [144].

For the treatment of acute promyelocytic leukemia, Tamibarotene (Am80) was loaded in a NLCs (Am80-NLC). The pegylated forms were prepared for intravenous delivery of Am80, with the purpose to further extend the circulation in blood and decrease the unfavorable events. Am80-loaded PEG-NLC (Am80-PEG-NLC) modified with PEG-40 stearate (PEG40-SA) was formulated by melt-emulsification and low temperature-solidification technique. *In vitro* drug release of Am80-NLC and Am80-PEG-NLC exhibited a sustained release characteristic. *In vivo*, after intravenous injection to rats, all the pharmacokinetics data were significantly prolonged and improved compared with the Am80-NLC group. In mice, the biodistribution of Am80-PEG-NLC was shown that preferentially decreased the accumulation of Am80 in kidney and increased the drug concentration in brain after intravenous injection. Then, it was concluded that Am80-PEG-NLC may be a potential delivery system for Am80 in the treatment of APL [145].

A chitosan (CS) coated nanostructured lipid carrier formulation (CS-NLC) (ultrasonication, Precirol ATO5 or Dynasan 114 and Miglyol, Tween 80 and Poloxamer 188 were used) was prepared for brain delivery after being intranasally administered. The *in vitro* assays demonstrated the biocompatibility of the formulation, the cellular uptake by 16HBE14o-cells and the absence of toxicity on erythrocytes. Biodistribution of CS-NLC-DiR demonstrated an efficient brain delivery of the particles after intranasal administration of C57 mice. Finally, CS-NLC was safe and effective nanocarrier for nose-to-brain drug delivery [146].

To design and evaluate the potential of a topical delivery system for ocular administration of voriconazole (VOR), cationic nanostructured lipid carriers with the drug were developed (VOR-NLCs) (VOR, glyceryl behenate/capric caprylic triglyceride, polysorbate 80, sorbitan trioleate, and cetylpyridinium chloride by microemulsion technique). *Ex vivo* ocular experiments proved that VOR-NLCs could deliver therapeutically relevant drug amounts to the cornea after only 30 minutes [147].

Engineered nano-drug delivery system for co-encapsulating paclitaxel (PTX) and doxorubicin (DOX) by the melt-emulsification method was developed (PTX-DOX-NCL). PTX and DOX were dissolved dimethylsulfoxide (DMSO), and then added to the hot lipid phase consisted of COMPRITOL 888 ATO, oleic acid and soybean phosphatidylcholine. Aqueous phase was prepared by dissolving N-[1-(2,3-dioleyloxy) propyl]-N,N,Ntrimethyl-ammonium chloride (DOTMA) in distilled water. PTX-DOX-NCL achieved the highest cytotoxic effect on NCl-H460 human non-small cell lung carcinoma cells (NSCLS). *In vivo* (BALB/c nude mice) investigation on NSCLC animal models showed that co-delivery of PTX and DOX possessed high tumor-targeting capacity and strong antitumor activity [148].

It was reported an increased curcumin (CUR) accumulation in the brain when formulated as CUR-NLCs (lipids precirol (solid lipid) and capmul MCM (liquid lipid), Tween 80, CUR and soya lecithin (SL) were chosen as a surfactant and a stabilizer, respectively) after being intranasally administered to rats. An increased cytotoxicity of CUR-NLC than that of CUR to astrocytoma-glioblastoma cell line (U373MG) in the cancer cell lines was observed. Results of biodistribution studies showed higher drug concentration in brain after intranasal administration of CUR-NLCs than e plain drug suspension (CUR). The authors suggested that CUR-NLC was a promising drug delivery system for brain cancer therapy [149].

NLC-Asenapine maleate (ASM) for the delivery of drugs in the brain by an intranasal route to enhance therapeutic efficacy was prepared. A brain pharmacokinetic study indicated a significantly higher peak drug concentration, area under the drug concentration-time curve (AUC0) and mean residence time (MRT of NCL-ASM compared to ASM in the brain via an intranasal route. The results of behavior studies of NLC-ASM showed a significant decrease in extra-pyramidal side effects with increasing antipsychotic effect after 1–2 week(s) of treatment. These results are indicative that NCL-ASM could be a promising drug delivery system for intranasal delivery of asenapine in the treatment of schizophrenia [150].

Nepafenac (NP)-NLC (melt-emulsification method with NP, glycerin monostearate, miglyol, soy lecithin and Cremophor EL) was prepared to apply after cataract surgery for the treatment of postoperative inflammation. The results of cytotoxicity studies showed that NLC-Gel was biocompatible with no significant cytotoxicity observed in the human corneal epithelial cells (HCECs). Preliminary cellular uptake tests proved an enhanced penetration of NP into HCECs when encapsulated in NLCs. Mechanistic study indicated that cell uptake was dependent on energy and the clathrin-mediated pathway. All data were indicative that the NP-NLC-Gel could be developed as a preliminarily carrier for delivering NP for the inflammation treatment [151].

Conclusions and future perspectives

NLCs among other lipids nanoparticles exhibit many advantages compared to existing nanoparticulated drug delivery systems. Maybe the most important aspect of these nanoparticles is that they are made of surfactants and lipids that are approved by the FDA and/or EMA, organic solvent absence, easily processes, inclusive in large batch by high pressure homogenization and are commercially available in marketed products, mainly for oral, dermal and intravenous administration [118].

Most of the researchers agree that the continuous improvement in the biomedical field and increased studies on transport mechanisms of NCLs by different administration route, both are important in the next step to successful results. Table II shows the main properties, advantages and some disadvantages that need to be solved, of lipid nanoparticles.

Table II. Main characteristics of hybrid solid lipid nanoparticles

Evidently from the present review there are many potential industrial applications of SLN/NLC, and this is reflexing in the publication of patents for different applications. Interesting reviews by different authors involving NCLs patents, describing critically this activity in this field were published [28,34,152–154].

It is notoriously that the increasing number of patents and publications with new studies, it will be that clinical trials in this area being increased in the next years leaving these studies closers to a clinical application of NCLs.

ABBREVIATIONS

ALI = Lung injury model
ASM = Asenapine maleate
BSA = Bovine serum albumin
CNS = Central nervous system
CS = Chitosan
CUR = Curcumin
DMSO = Dimethylsulfoxide
DOPE = 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine
DOX = Doxorubicin
DSC = Differential Scanning Calorimetry
DSPE = 1,2-distearoyl-snglycero-3-phosphoethanolamine
DSS = Dextran-sulfate
HCECs = Human corneal epithelial cells
HCPT = Hydroxycamptothecin
HPMEC = Human Pulmonary Microvascular Endothelial cells
IN = intranasal
IP = intraperitoneal
IV = intravenous
LHRH = Luteinizing hormone-releasing hormone
LPNs = Lipid-polymeric nanoparticles

NLC = Nanostructured Lipid Carriers
NP = Nepafenac
NSCLS = Non-small cell lung carcinoma
OND = Ondansetron
PEG = Polyethylene glycol
PEI = Polyethylenimine
PLGA = Poly-lactide-co-glycolide acid
PTX = Paclitaxel
RGD = Tumor-targeting peptide
RP = Ropinirole
SEM = Scanning Electron Microscopic
SLN = Solid Lipid Nanoparticles
SQV = Saquinavir
SQV = Saquinavir
VOR = Voriconazole
XDR = X-ray diffraction
ZER = Zerumbone
β-E = β-Elemene

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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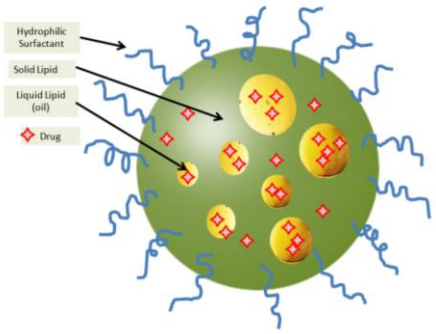
Figures

Figure 1 Nanostructure lipid carriers (NLC). Scheme of drug loaded NLC (a); characterization of NLC particle size distribution by transmission electron microscopy (TEM) images (b). Figure obtained from **Islan et al., 2016** with permission.

a



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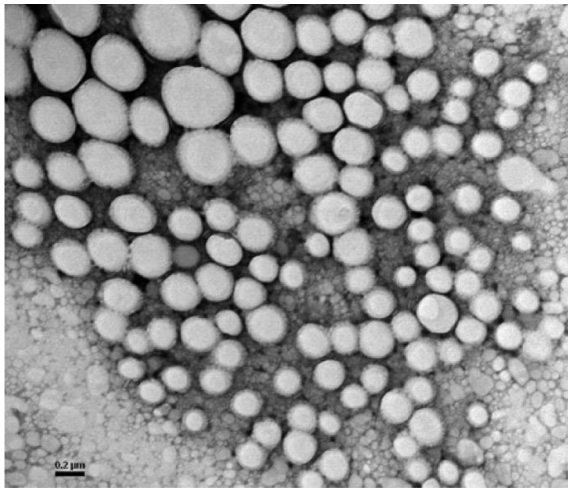
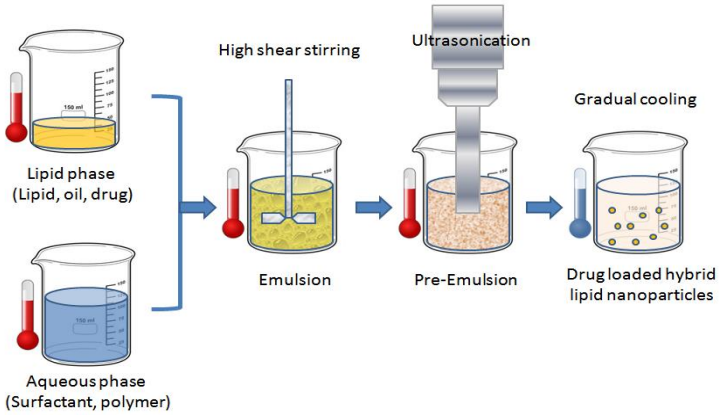


Figure 2 Scheme of the most used methods for preparation of one type of hybrid lipid nanoparticle: a) emulsification/ultrasonication; b) solvent diffusion method.



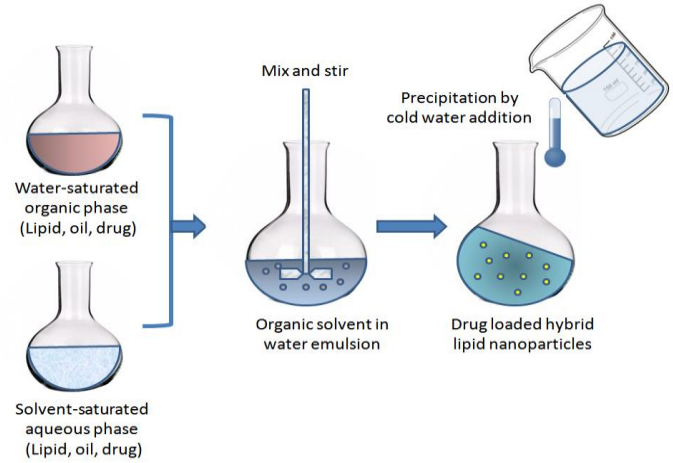


Figure 3 Models for lipid-polymer hybrid nanoparticles: a) polymer core and lipid shell; b) lipid core and polymer coating. Drugs accommodate within the matrix according to the molecular nature.

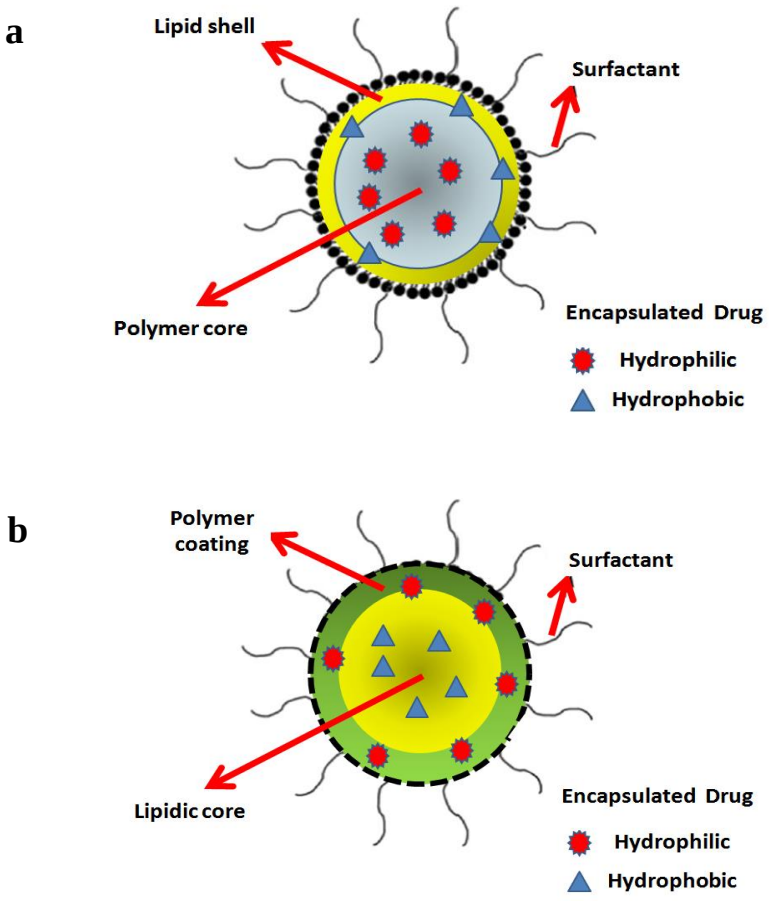


Table I. LPNs with active targeting moieties

		Doxorubicin with sorafenib	iRGD	xenograft tumor model (HepG2)	Zhang et al., 2016
PLGA	DSPE	Doxorubicin	Folate	MCF7	Zheng et al., 2015
PLGA	DSPE-PEG2000-COOH	Curcumin	RNA Aptamers against EpCAM	HT29	Li et al., 2014
PLGA	DSPE-PEG2000-COOH	Vinorelbine	MUC aptamer	MCF7 and HepG2	Liu et al., 2015
PLGA	DSPE-PEG2000-COOH	Paclitaxel	anti-carcinoembryonic antigen (CEA) half-antibody	BxPC-3 and XPA-3	Hu et al., 2010
PLGA	stearylamine	Amphotericin B	stearylamine	*L. donovani* amastigotes infected hamsters	Asthana et al., 2015
PLGA	DOPE	7-APTADD	Transferrin	SKBR-3	Zheng et al., 2010
mPEG–PLGA	cholesterol	Curcimin	RGD	B16 melanoma tumor model	Zhao et al., 2014

See abbreviations section.

Table II. Main characteristics of hybrid solid lipid nanoparticles

Hybrid solid lipid nanoparticles properties	
Advantages	**Disadvantages**
Enhanced structural stability	Nanoparticle growth
Enhanced drug loading	Gelation trends
Enhanced drug activity and stability	Unpredictable interactions between matrix components and the payload
Defined kinetic drug release	Burst release of hydrophilic drugs
Transport of hydrophilic and hydrophobic molecules	Difficult to obtain homogeneous dry powders
Feasible tailoring and targeting using Green Chemistry approaches	
High biodegradability	
Low toxicity	
Aqueous synthesis (avoiding organic solvents)	
Feasible scale up	
Less expensive than chemical synthesized systems	