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REVIEW

A Review on QbD-Driven Optimization of Lipid Nanoparticles for Oral Drug Delivery: From Framework to Formulation

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Abstract: Oral administration is the most preferred route for drug delivery due to its convenience, non-invasiveness, and patient compliance. However, it is challenged by gastrointestinal barriers, enzymatic degradation, and first-pass metabolism, which reduce drug bioavailability. Lipid nanoparticles (LNPs), including solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), offer a promising strategy to overcome these limitations by enhancing drug stability, permeability, and absorption. The Quality by Design (QbD) framework provides a systematic approach for LNP development to ensure consistent product quality. By promoting process understanding and control, QbD not only supports scientific formulation development but also enhances industrial scalability by reducing the experimental workload, shortening the development time, and lowering the production costs. This review highlights key QbD elements such as the quality target product profile (QTPP), critical quality attributes (CQAs), critical material attributes (CMAs), critical process parameters (CPPs), and design of experiments (DoE) and their roles in guiding formulation and process optimization. The effects of various CMAs and CPPs on the CQAs such as particle size, polydispersity index, encapsulation efficiency, zeta potential, and drug release are discussed. Furthermore, the in vitro, in vivo, and ex vivo performances of optimized LNPs were explored in detail. Overall, QbD offers a robust platform for the rational design and scalable production of high-quality lipid-based drug delivery systems for oral administration.

Keywords: quality by design, optimization, solid lipid nanoparticles, nanostructured lipid carriers, oral administration

Introduction

Nanotechnology offers broader opportunities for the development of novel drug delivery systems to overcome the limitations of traditional pharmaceutical formulations. Conventional drugs suffer from drawbacks such as poor solubility and low permeability, which subsequently result in poor bioavailability and therapeutic efficiency.[1] These issues are particularly pronounced in oral drug delivery, owing to environmental variability, enzymatic degradation, and first-pass metabolism.[2] Nanotechnology-based drug delivery systems exploit their size and specific structure to circumvent these hurdles. Various nanocarriers, including micelles, dendrimers, polymer-based nanosystems, and lipid-based nanosystems, have been developed to enhance the effectiveness of drug delivery via various routes.[3–5] Lipid-based nanosystems, which are often categorized as vesicular systems, such as liposomes, niosomes, ethosomes, transferosomes, and matrix systems (or lipid nanoparticles, LNPs), such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), have attracted considerable interest

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owing to their potential as drug delivery systems.[6–9] In oral drug delivery, LNPs can protect the drug from degradation in the acidic environment of the stomach and enzymatic hydrolysis of the intestines.[10,11] They also proved to be effective in enhancing permeability across epithelial membranes, facilitating lymphatic transport, and bypassing first-pass metabolism, thereby improving systemic bioavailability.[12–14]

Despite their potential as novel drug delivery systems, a comprehensive understanding in LNPs production remains lacking. The influences of input materials and process parameters on product properties and therapeutic performance have not been clearly explained, which can be attributed to inconsistencies such as batch-to-batch variations in particle size, zeta potential, encapsulation efficiency, and drug release profiles during formulation. Furthermore, the traditional experimental procedure of changing one-factor-at-a-time (OFAT) is deemed inefficient.[15] For instance, modifying the lipid type without adjusting surfactant concentration may not improve drug loading, and important interactions between formulation variables may be overlooked. Correspondingly, it was realized that adding more tests did not enhance the product quality. The trial-and-error nature of this method is also resource-intensive, as researchers often need to restart the entire experiment when target quality attributes are not achieved. This approach poses challenges not only in terms of cost, but also in meeting predefined product quality requirements.[16,17]

The quality by design (QbD) approach offers a more systematic design for production, reducing exhaustive experimental procedures to more efficient ones. The QbD strategy for pharmaceutical formulations has been implemented since the publication of several guidelines by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH).[18–23] The foundation of QbD is based on scientific design and manufacturing. Its core value is that the quality should be incorporated into the product. In addition to pharmaceutical knowledge, a mathematical-statistical understanding is important when applying QbD elements in pharmaceutical production. As part of QbD, optimization via design of experiment (DoE) is crucial for defining the mathematical model of the relationship between independent and dependent variables. DoE can also identify the statistical significance and optimal conditions to achieve the desired drug quality.[24]

In this review, the challenges of oral administration and the corresponding role of LNPs are discussed. Subsequently, we explored the implementation of QbD principles by identifying the quality profile in relation to the selection of materials and preparation methods based on risk assessment to understand the path for improvement in SLN and NLC production for oral drug delivery. Additionally, this article summarizes the findings from recent studies regarding the impact of input variables on output responses based on DoE results, where the optimized products were evaluated in vitro, in vivo, and ex vivo for various purposes.

Challenges of Oral Drug Delivery

Oral delivery is generally considered a more favorable drug administration route for patients and physicians owing to its effortlessness, noninvasive nature, and high patient acceptability.[25] Additionally, orally administered drugs may specifically target certain regions, thereby localizing therapy in gastrointestinal (GI) diseases, such as gastroesophageal reflux disease, inflammatory bowel disease, GI cancer, and colorectal cancer.[26–29] Despite these advantages, oral drug delivery still faces challenges associated with the inherent properties of drugs and the complexity of the GI system during their traversal.

The GI tract is particularly intriguing because of the variability in its environment. Greatly different pH values are encountered by the drugs, from acidic conditions in the stomach (pH 1 − 2.5), duodenum (pH 6.1), intestines (pH 7.1 − 7.5), and higher pH conditions in the colon (pH 7 − 8).[30] Furthermore, the presence of various GI enzymes is particularly challenging for protein-based drugs. They may be hydrolyzed and degraded by pepsin in the stomach or by other proteolytic enzymes in the small intestine.[31] In contrast, the enzymatic activity of pancreatic lipase may promote lipolysis in the GI tract and enhance the solubilization of lipophilic drugs or lipid-based formulations.[32] GI enzymes also play pivotal roles in the first-pass metabolism. This presystemic metabolism is mediated mainly by cytochrome P450 enzymes such as CYP3A4, which are predominantly found in the liver and small intestine.[33] The large surface area and low blood flow in the small intestine might prolong CYP3A4 exposure toward drugs, enabling more extensive metabolism and thus decreasing the oral bioavailability of the drugs.[34] This intestinal first-pass effect is markedly enhanced by drugs that are substrates of CYP3A4, such as felodipine, nifedipine, atorvastatin, and simvastatin.[35]

The small intestine is considered the primary site of oral drug absorption, owing to its extensive surface area and various transport modes. The microvilli in the small intestine are lined with goblet cells, which facilitate glycoprotein secretion and form a mucosal layer.[36] This layer is primarily composed of mucin, an oligosaccharide-rich glycosylated protein that provides an overall negative charge to mucus, thus facilitating electrostatic interactions with positively charged substances.[37,38] Intestinal mucus is also involved in the formation of an unstirred water layer (UWL) between the intestinal bulk fluid phase and the epithelial brush border. The presence of UWL can be detrimental to lipophilic substances because of hindered access to the epithelium, limiting their passage to the systemic circulation.[39]

To reach the systemic circulation, drugs must be able to cross the intestinal epithelium, which primarily comprises enterocytes, along with a smaller number of other cells, such as goblet cells, microfold cells (M cells), and Paneth cells.[40] Epithelium crossing involves several different transport mechanisms owing to its particular structure and constituents. Passive diffusion is the most common pathway, which relies on the movement of drugs along their concentration gradient, from a higher amount on the apical side to a lower amount on the basolateral side of the membrane. This mechanism may occur via the paracellular or transcellular routes. In the paracellular route, drugs traverse the enterocytes through intercellular tight junctions, allowing the movement of smaller hydrophilic molecules. In contrast, lipophilic molecules can naturally diffuse through the cell membrane transcellularly, owing to their similar affinities.[41] Furthermore, transcellular absorption may also occur via endocytic mechanisms, such as phagocytosis by immune cells or M cells, macropinocytosis, clathrin-mediated or clathrin-independent endocytosis, and caveolae-mediated or caveolae-independent endocytosis.[42]

Unlike passive diffusion, active transport allows molecules to move against the concentration gradient. This transporter-mediated movement offers specific pathways during drug traversal and requires a certain amount of energy derived from adenosine triphosphate (ATP). Among the ATP-dependent transporters, the role of P-glycoprotein (P-gp) has been particularly highlighted in oral drug administration. P-gp is an efflux pump that acts as a defense mechanism by actively pushing xenobiotics out of enterocytes.[36] However, this mechanism can be disadvantageous for orally administered drugs because it reduces the intracellular concentration of drugs, thereby limiting drug absorption into systemic circulation and reducing oral bioavailability. This limitation is particularly pronounced for the absorption of drugs that are P-gp substrates.[43]

Role of Lipid Nanoparticles in Oral Drug Delivery

Drug incorporation into LNPs, such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), is a feasible strategy for overcoming the variability of GI barriers and the complexity of transport modes during oral administration (Figure 1). As a nanoscale system, the structure of LNPs intrinsically generates a larger surface area. This results in increased interaction between the systems and biological membranes, facilitating enhanced absorption into blood circulation.[44] The components of LNPs, which mainly comprise lipids and surfactants as well as additional excipients, provide specific advantages for oral drug traversal.

Protection From Degradation

The solid lipid components in LNPs, particularly long-chain fatty acids, can slow the degradation process by digestive enzymes, resulting in higher drug stability in the GI environment.[40] This allows the drug to reach its target active form, thereby enhancing its therapeutic effectiveness.[45] A study conducted by Veni and Gupta showed that the drug release of linagliptin formulated into SLN using stearic acid as a solid lipid at pH 6.8 was higher than that at pH 1.2. This signifies the ability of stearic acid to protect linagliptin from the gastric environment, thereby ensuring intestinal release of the drug.[46]

Using cetyl palmitate as a solid lipid component in the LNP formulation can also be advantageous for minimizing drug degradation owing to its low susceptibility to lipase hydrolysis, leading to prolonged drug retention in the formulation.[47] El-Dakroury et al reported that the cumulative release of fexofenadine HCl in an acidic medium was lower when formulated in SLN using cetyl palmitate compared with the pure drug.[48] Similarly, the release of doxorubicin from NLC containing the same solid lipid was lower in simulated gastric fluid than in simulated intestinal fluid.[49]

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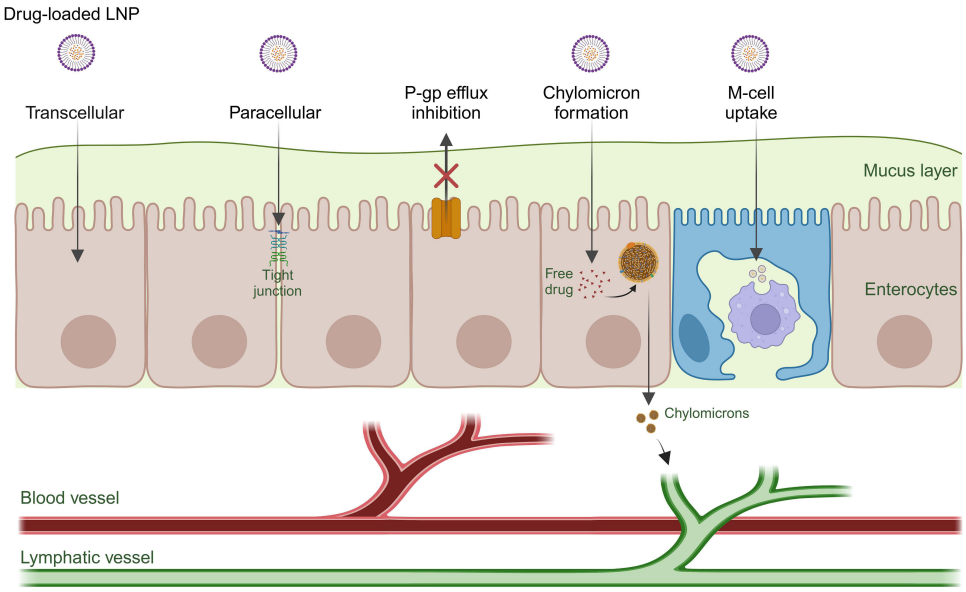

Figure 1 Drug transport mechanisms across intestine. Created in BioRender. Suliman, (K) (2025) https://BioRender.com/vmkadsf.

Inhibition of Cytochrome P450

First-pass metabolism, mainly mediated by cytochrome P450 enzymes, such as CYP3A4, is a major drawback of effective oral administration. Several LNP constituents may act as modulators of CYP3A4 in bypassing the first-pass metabolism, and subsequently increasing drug absorption.[50] Unsaturated fatty acids, such as oleic acid, can inhibit CYP isoforms including CYP3A4. This may be due to the ability of fatty acids to disrupt the microsomal membrane and prevent drug binding to the enzyme active site.[51] Several reports have shown that formulating NLC using oleic acid as a liquid lipid can improve the oral bioavailability of telmisartan and fexofenadine HCl.[52,53] In a study by Sharma et al, the use of oleic acid in NLC markedly increased the plasma concentration and bioavailability of atorvastatin, which is a CYP3A4 substrate, compared to the marketed drug.[54]

Surfactants also play a pivotal role in the inhibition of CYP3A4. Non-ionic surfactants, such as Cremophor® EL and Cremophor® RH-40 have inhibitory effects on CYP3A4, with IC50 values ranging between 0.40 to 0.80 mM. Cremophores can alter drug absorption owing to agent-produced membrane fluidization, causing perturbations toward the CYP3A4 microenvironment, thus decreasing enzyme function.[51] An in vivo pharmacokinetic study demonstrated increased bioavailability of silybin in SLN formulated using Cremophor® RH40 as a surfactant.[55] In another study, it was observed that using Cremophor® EL in the SLN of fenofibrate, a CYP3A4 substrate, resulted in higher plasma concentrations than those of the pure drug.[56]

Inhibition of P-Glycoprotein

P-glycoprotein (P-gp) is an ATP-binding cassette transporter predominantly found in the apical layer of epithelial cells. Its primary function as a xenobiotic efflux pump can be detrimental for oral delivery due to restricted drug transport across the basolateral layer, thereby limiting the amount of drug in the systemic circulation.[57] Designing LNPs with appropriately selected constituents that act as P-gp inhibitors is an effective strategy to improve drug transport across the intestinal barrier. In general, the commonly proposed P-gp inhibitory mechanism involves obstruction of drug-binding sites, disruption of ATP hydrolysis, and alteration of cell membrane integrity.[58] Lipid-based excipients, such as glyceryl monooleate and hard fat, as well as surfactants, such as Cremophor®, poloxamers, and polysorbates, are among the components that can be utilized in LNP formulations for these purposes.[59] Tween® 80 (polysorbate 80) has been specifically recognized for its synergistic role as an inhibitor of P-gp and CYP3A4.[60] Beloqui et al showed that the transport of saquinavir, a known P-gp substrate, across Caco-2 cells was enhanced when incorporated into an NLC system prepared with polysorbate 80.[61] Furthermore, an in vitro permeability study demonstrated higher apical-

basolateral transport of tilmicosin-loaded NLC prepared using poloxamer 188 and polysorbate 80 as surfactants.[62] The use of Gelucire® 44/14 and polysorbate 80 as liquid lipids and surfactants in NLC also significantly increased the plasma concentration of iloperidone, confirming the potential of both excipients as P-gp inhibitors.[63]

Enhancement of Lymphatic Pathway

The enhancement of oral bioavailability offered by LNPs can also be facilitated through lymphatic transport, allowing lipophilic drugs to bypass first-pass metabolism. Lipids in LNPs may promote the formation of chylomicrons, which are large lipoproteins that are partly responsible for transcellular drug absorption.[36] In the GI tract, triglycerides from lipids are broken down by lipase into monoglycerides and free fatty acids, which are then absorbed by enterocytes and incorporated into chylomicrons. These drug-loaded chylomicrons subsequently enter the lymphatic capillaries and avoid hepatic metabolism to finally reach the systemic circulation.[45] Molecules with Log P > 5 are naturally transported via the lymphatic pathway. However, drugs with lower Log P values, such as atazanavir (Log P = 4.1), can also utilize this route when encapsulated within the NLC system. Using the chylomicron flow block model, Gurumukhi and Bari revealed the ability of NLC to circumvent first-pass metabolism, resulting in a higher plasma concentration of atazanavir.[64]

Lymphatic transport can also be facilitated transcellularly through uptake by M cells, which are specific epithelial cells essential for the intestinal immune system. These cells are primarily found in the gut-associated lymphoid tissue (GALT) or Peyer's patches.[40] Functionalization of LNPs with targeting ligands such as lectins enables specific binding to receptors on the M cell surface, thereby enhancing absorption and subsequent transport into the lymphatic system. A study by Hädrich et al demonstrated increased phagocytosis of quercetin NLC when its surface was functionalized with wheat germ agglutinin.[65]

Enhancement of Mucoadhesion

LNPs can also contribute to enhanced mucoadhesion in oral drug delivery via electrostatic, covalent, hydrogen, and van der Waals interactions.[66] Intestinal epithelial cells are protected by a hydrophilic negatively charged mucus layer that serves as a barrier against foreign particles. The functionalization of LNP using positively charged polymers, such as chitosan, is considered a feasible strategy to enhance mucoadhesion via electrostatic interactions, subsequently prolonging the drug residence time in the GI tract, enabling controlled drug release, and improving oral bioavailability. A study by Pyo et al showed higher plasma concentrations of chitosan-coated fenofibrate NLC than of uncoated fenofibrate NLC. The authors suggested that chitosan contributes to enhanced mucoadhesion while also acting as a tight junction modulator in intestinal enterocytes, thus facilitating drug transport into the systemic circulation.[67] In another study, a chitosan-functionalized SLN of thymoquinone showed a higher mucoadhesive efficiency than that of free thymoquinone. This may be due to electrostatic interactions between the cationic chitosan-functionalized SLN and anionic mucin molecules. In addition, the hydrophilic properties of chitosan further intensified mucoadhesion.[68]

Mucoadhesion can also be enhanced through covalent bonding between mucosal cysteine residues and thiomer (thiolated polymer) molecules. For instance, it was found that the use of thiolated polyoxyethylene oleyl ether surfactant in aprepitant-loaded NLC displayed prolonged adhesion to goat intestinal mucosa compared to unmodified surfactant-based aprepitant NLC and aprepitant suspensions. Furthermore, the modified NLC formulation also exhibited increased plasma concentration and relative oral bioavailability compared to the drug suspension and unmodified NLC.[69]

Quality by Design Framework

The development of LNPs involves complex interactions between formulation components and process parameters, which often result in challenges such as variability in particle size distribution, low encapsulation efficiency, poor drug loading, low zeta potential, and inconsistent drug release behavior. These attributes are critical to the performance of LNPs but are difficult to optimize using traditional OFAT approaches because of the absence of interaction analysis and the need for numerous trial-and-error iterations. In this context, the QbD framework serves as a powerful and structured approach for systematically exploring the formulation space. By employing design of experiments (DoE), QbD enables the identification and control of critical material attributes (CMAs) and critical process parameters (CPPs) that influence the critical quality attributes (CQAs). Beyond its conceptual strengths, QbD also offers practical advantages such as

shortened development time, reduced experimental workload, and enhanced precision in targeting desired product characteristics. This is particularly valuable for complex systems like SLNs and NLCs, where small variations in formulation or processing can significantly impact particle size, encapsulation efficiency, and release profile, which subsequently translates into therapeutic performances. Consequently, QbD not only improves formulation robustness and scalability but also supports regulatory alignment and cost-effective LNP development.[70,71]

Quality Target Product Profile

Quality target product profile (QTPP) is a foundational component of the QbD framework, outlining the desired profile of a drug product to provide optimal safety and efficacy.[70] In pharmaceutical product development, the QTPP provides a prospective summary of the final drug product, including dosage form, delivery system, route of administration, dosage strength, container closure system, drug release, pharmacokinetic properties, purity, sterility, and stability.[18] In the context of oral drug delivery, the identification of QTPP related to the enhancement of systemic and/or lymphatic absorption is particularly crucial.[72] For example, in a QbD-based study of atazanavir-loaded NLC, the QTPP stated that the defined pharmacokinetic parameters should be higher than the reference to provide higher drug concentrations, ensuring higher lymphatic uptake of the drug.[64]

In the identification of the QTPP, researchers should consider the regulatory requirements for bioequivalence and patient adherence, ensuring that the product matches the therapeutic performance of the reference products.[73] The QTPP not only guides formulation and manufacturing strategies but also provides a benchmark for assessing critical quality attributes (CQAs) throughout the development process, aiming for a high-quality pharmaceutical product that meets both safety standards and therapeutic goals.

Critical Quality Attributes

As defined in the ICH Q8 (R2) document, CQA is another important element of QbD that represents the physical, chemical, biological, or microbiological properties of a drug product, which must remain within a defined range, limit, or distribution to ensure the desired quality.[18] Quality attributes can be either critical or noncritical. When failure to achieve a specified range results in no efficacy or potential harm to the patient, an attribute should be considered critical.[73] Along with the QTPP, CQAs play a vital role in guiding product and process development, ensuring that the safety and efficacy standards are met. However, CQAs differ from QTPP in scope and function within the QbD framework. QTPP outlines the overall desired characteristics and quality of the final drug product, such as the expected release profile and therapeutic effect, whereas CQAs may include specific parameters, such as particle size and encapsulation efficiency, which need to be tightly controlled to meet the target profile.[74] Furthermore, unlike the more fixed profile of the QTPP, CQAs serve as adjustable responses to changes in the formulation attributes or process parameters. Thus, CQAs play a critical role in bridging the quality objectives outlined in the QTPP with the practical aspects of formulation and process development.[71]

In the production of lipid-based nanocarriers, common CQAs include particle size, polydispersity index, encapsulation efficiency, drug-loading capacity, cumulative drug release, and zeta potential.[75,76] For orally administered drugs, the control of uniformly small particles is substantial because nanosized particles (<1000 nm) provide a greater surface contact area, subsequently increasing the intestinal absorption.[77] Furthermore, nano-sized particles can be transported both paracellularly and transcellularly (via endocytosis by enterocytes or via M cell uptake).[25,78] However, a larger particle size may be useful for extended drug release. LNPs larger than 150 nm are more likely to be taken up by phagocytes, which act as reservoirs and accumulate inside the liver or spleen over an extended period before being gradually released into systemic circulation.[79,80]

The selection of zeta potential as a CQA also affects the performance of the final product. Zeta potential describes the surface charge of a colloidal particle, which is measured as the electrical potential at a layer relative to a certain point in the bulk medium. A higher absolute value of the zeta potential ($\geq \pm 30$ mV) indicates stronger repulsive electrostatic interactions between particles, thus preventing aggregation and ensuring the stability of the dispersion system.[81] Moreover, from the perspective of drug delivery, the surface charge of nanoparticles is partially responsible for stronger membrane binding and cellular uptake enhancement.[82,83] Nisini et al found that positively charged liposomes could interact with the negatively charged mucosal surfaces of tumor cells, facilitating liposome endocytosis by antigen-

presenting cells, thus enhancing cell-mediated immune responses.[84] The dependence of cytotoxicity on the zeta potential was also demonstrated in a study by Shao et al, where positively charged nanoparticles resulted in higher cytotoxicity toward L929 cells.[85]

Another property that may be considered a CQA in orally administered LNPs is the percentage of unpleasant taste, which describes the palatability of the drug product. In a study of diacerein-loaded SLN, it was found that an optimum amount of lipid was suitable for producing a palatable preparation.[86] Several studies have also revealed the ability of lipids to control the release of bitter drugs in saliva, effectively sustaining the concentration of drugs that reach bitter taste receptors.[87,88]

Critical Material Attributes

Critical material attributes (CMAs) mainly identify the state of the input materials, such as the drug substances and excipients employed during production. These attributes encompass a wide range of material properties within an acceptable range, which can influence the quality profile of the final product.[89] Determining the interrelationship between CMAs and CQAs is fundamental during the QbD process, where material attributes are systematically identified, screened, and controlled based on their impact on quality.[90] In the development of SLNs and NLCs for the oral route, determination of CMAs is particularly crucial in relation to their objectives, both to protect the drugs from the GI environment and to deliver them into the systemic circulation.

Generally, the type and amount of lipids are the primary considerations in LNPs production. Changes in the type of lipid, drug-to-lipid ratio, solid-to-liquid lipid ratio, or total lipid concentration can affect the particle size, drug encapsulation efficiency, and release profile of nanoparticles.[62,91] For example, the use of solid lipids in different polymorphs can influence the phase transition temperature, which affects lipid crystallinity and the likelihood of drug expulsion from the nanoparticle matrix.[92] In NLCs, the optimum amount of liquid lipids may provide greater encapsulation efficiency because of the lower melting point of the system, which subsequently enhances the dissolving capacity of the matrix.[93] Furthermore, solid and liquid lipid compositions also affect the type of NLCs, which are classified as imperfect, amorphous, or multiple NLCs. In imperfect NLCs, a lower amount of liquid lipid is blended with solid crystalline lipids, such as glycerides, reducing crystallinity and promoting the formation of an unstructured matrix during cooling. This facilitated a higher drug loading inside the matrix voids.[94] In the amorphous type, specific non-crystalline solid lipids, such as medium-chain triglycerides, hydroxystearate, or isopropyl myristate, form an amorphous core along with liquid lipids. The disordered nature of the matrix can minimize drug leakage during storage.[95] In multiple NLCs, liquid lipids such as medium-chain and long-chain triglycerides or oleic acid are employed in higher amounts, which enables phase separation and oil compartment formation within the solid lipid. This compartment provides a suitable environment for solubilizing lipophilic drugs, and subsequently promotes sustained or controlled drug release during oral administration.[96]

Surfactants also play an important role as CMAs in LNPs formulation. The type and concentration of the surfactant can modify the surface properties of the nanoparticles, thereby influencing drug loading, stability, particle size distribution, and pharmacokinetic profile.[97] For instance, cationic surfactants such as hexadecyltrimethylammonium bromide (CTAB) can improve mucoadhesion by forming electrostatic interactions with negatively charged endocytosis-inducing biological membranes, thus enhancing the cellular uptake of nanoparticles.[98,99] Both the lipids and surfactants selected in the SLN and NLC formulations should be generally recognized as safe based on their biocompatibility, biodegradability, and non-toxicity (Table 1).

In surface-modified LNPs, the coating materials can also be considered CMAs. A study by Veni et al indicated that increasing the amount of eudragit, a pH-sensitive polymer, delayed the drug release from the SLN matrix. Eudragit-coated SLN remain stable in acidic gastric environments, whereas the drug is gradually released upon arrival in alkaline intestinal environments.[46] The addition of surface charge modifiers to LNPs may also be beneficial. El-say et al reported that a higher zeta potential was obtained by increasing the concentration of stearylamine, a positive charge-inducing agent, thus improving the stability of LNP.[123]

Critical Process Parameters

Critical process parameters (CPPs) refer to specific input operating or process state variables that are controlled and monitored during production.[89] These parameters can impact CQAs, and highly impactful factors should be prioritized.

Table 1 Common Excipients in LNPs for Oral Drug Delivery

Component	Substance	Ref.
Solid Lipid	Behenoyl polyoxyl-8 glycerides (Compritol® HD5 ATO)	[13]
	Cetyl alcohol	[86]
	Cetyl palmitate	[48,49]
	Glyceryl behenate	[56,100–108]
	Glyceryl monooleate	[91,109]
	Glyceryl monostearate	[52,68,110–122]
	Glyceryl palmitostearate	[56,123–127]
	Hard fat	[49]
	Mono and diglycerides (Geleol® mono and diglyceride NF)	[64,128]
	Phospholipon® 90G	[129]
	Soy lecithin	[130]
	Stearic acid	[46,53,55,62,131,132]
	Tripalmitin	[133]
Liquid Lipid	Capric and caprylic acid triglyceride	[130]
	Caprylocaproyl polyoxyl-8 glycerides	[115]
	Linoleoyl polyoxyl-6 glycerides	[124]
	Linseed oil	[127]
	Medium chain mono and diglycerides	[116,117]
	Oleic acid	[49,52,53,62,91,102,104,111,113,121,131,132]
	Propylene glycol dicaprylate	[128]
	Propylene glycol monocaprylate	[13,64,103,119,120,133]
	Sucupira Oil	[122]
	Triglycerides	[118]
	Vitamin E	[125,126]
Surfactant	d-α-tocopheryl polyethylene glycol succinate (Vitamin E TPGS)	[114,122]
	Egg lecithin	[107]
	Ethoxylated castor oil (Cremophor® EL)	[56]
	Hydrogenated castor oil (Cremophor® RH 40)	[55]
	Lauroyl polyoxyl-32 glycerides (Gelucire® 44/14)	[123]
	Pluronic® F127	[13,118,119]
	Pluronic® F68	[46]
	Poloxamer	[68,91,105,107,110,116,120,125]
	Polyethylene glycol 12-hydroxystearate (Solutol® HS15)	[124,130]
	Polyoxyl 40	[127]
	Polysorbate 20	[48,52,115,117]
	Polysorbate 80	[49,86,100–102,106,111,113,121,132]
	Sodium lauryl sulfate	[53]

The criticality of a process parameter lies in its ability to satisfy the desired product quality. In LNPs production, the identification of potential CPPs is directly related to the selection of the preparation method (Figure 2). Based on preliminary information, the operating range of a specific parameter can be established to obtain the optimal conditions for the preferred CQA results.[70] Several preparation methods, such as melt emulsification-ultrasonication, high-pressure homogenization (HPH), and high-shear homogenization (HSH), have been widely employed for the QbD-driven development of LNPs.[100,110,111] Other methods such as hot-melt extrusion, phase inversion temperature, solvent diffusion, solvent evaporation, and spray drying have also been reported.[91,130,134,135] However, there have been fewer QbD studies conducted using these methods.

Each preparation technique offers a different mechanism along with its advantages and disadvantages. For example, in HPH, input materials are accelerated by high pressure (100 − 2000 bar) through a micron-size gap, where the resulting cavitation force and shear stress can break down the particle to the nanometer size.[136] However, the high-energy nature of HPH potentially leads to a suboptimal polydispersity index (PDI) owing to uneven particle disruption.[64] It is worth noting that process variations in HPH involving hot and cold conditions facilitate distinctive LNP characteristics. In hot HPH, the drug-lipid melt is combined with a surfactant above the lipid melting point. The mixture was emulsified. The hot emulsion was subsequently homogenized for several cycles at a specific pressure and cooled to room temperature to obtain the SLNs or NLCs. Conversely, in cold HPH, the

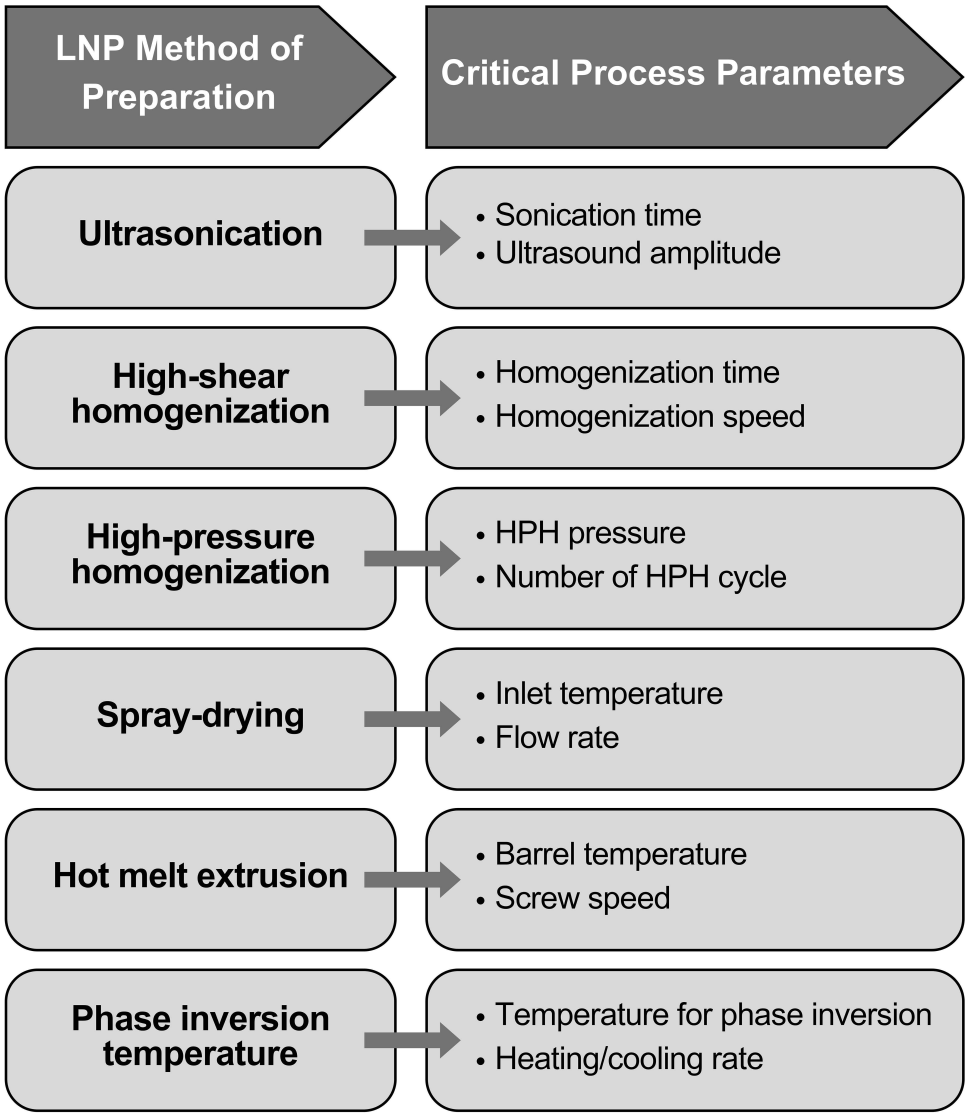

Figure 2 Critical process parameters in various LNP preparation methods.

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drug-lipid melt is first solidified using dry ice or liquid nitrogen. The solid was then crushed to obtain micron-sized particles, which were dispersed in a cold surfactant solution, followed by high-pressure homogenization.[137] Relatively smaller particles can be obtained using the hot process because of the decreased viscosity at higher temperatures. Nevertheless, high temperatures may also promote rapid degradation of the LNP system;[136] thus, certain conditions should be optimized to control the quality of the final product. During the HPH process, the number of homogenization cycles also contributes to the particle size and PDI and is commonly considered a CPP in the HPH technique.[138]

High-shear homogenization and ultrasonication-based methods are other approaches involving high energy. Both techniques offer a similar mechanism. Prior to nanosizing, the molten lipid was prepared at 5 − 10 °C above its melting point and dispersed in a surfactant solution under stirring at the same temperature. The resulting emulsion was homogenized or sonicated to reduce the droplet size to the nanometer scale.[139] The homogenization speed and cavitation-generating ultrasound amplitude are responsible for particle breakdown and thus may be considered as CPPs in their respective methods.[140] Concurrently, the duration of sonication and homogenization should be optimized to obtain the desired LNP characteristics without overheating the sample.[141]

In the spray-drying method, LNPs can be converted into dry powders that offer better physicochemical stability than the dispersion form. Atomization of the aqueous LNP dispersion may occur because of the high inlet temperature of the spray dryer.[142] The feed flow rate also promotes physical quality alteration of the LNPs. Mozaffar et al reported that, at a higher temperature (180 °C) and flow rate (15 mL/min), the particles were more evenly distributed and free of aggregates.[135]

The hot-melt extrusion (HME) technique is a relatively less explored method for LNPs preparation, despite its scalability and environmentally friendly characteristics.[143] During HME, the main ingredients are pumped and mixed inside an extruder barrel at 10 − 15 °C above the solid lipid melting temperature. Excipients can be subsequently added to certain feeding zones during extrusion. The resultant hot pre-emulsion was then subjected to either sonication or high-pressure homogenization to reduce the particle size.[56,134,144] In this method, varying the extrusion barrel temperature and screw speed evidently influenced the particle size and encapsulation efficiency of the LNPs, and thus could be selected as CPPs during production.

In the phase-inversion temperature (PIT) method, a certain type of emulsion is transformed into its reversed type by continuously changing the mixture temperature.[136] This technique primarily exploits the temperature-dependent properties of the hydrophilic-lipophilic balance (HLB) of surfactants. A mixture of drugs, lipids, and surfactants was prepared prior to the phase inversion. The emulsion was then subjected to several cycles of heating and cooling, followed by rapid cooling using cold water (0 °C). This treatment breaks down the emulsion system, resulting in the formation of stable LNPs.[130] The temperatures for phase inversion and the heating/cooling rate may influence the resulting nanoparticles; thus, they can be considered as CPPs in the PIT method.[145]

To select CPPs for each preparation technique, it is necessary to recognize the tunable operating parameters and process state variables that influence the CQAs of the product. Subsequently, the established potential operating space could be further employed during continuous manufacturing of LNPs.

Risk Assessment

In the QbD framework, risk assessment is a vital yet distinct component that supports decision-making by identifying and prioritizing material attributes and process parameters that may impact CQAs.[18] While QbD focuses on defining and achieving the QTPP through the systematic control of formulation and process variables, risk assessment serves to anticipate potential sources of variability or failure that could compromise product quality, safety, or cost efficiency.

Several risk assessment tools can be used to guide this process. For example, the Ishikawa (fishbone) diagram provides a broad overview of the contributing factors from each category, describing the cause-and-effect relationships between the variables (Figure 3).[112,124] The risk estimation matrix (REM) ranks variables based on their qualitative impact on CQAs.[101] In contrast, quantitative tools like failure mode and effects analysis (FMEA) assign numerical values to severity, occurrence, and detectability of potential failures to generate a risk priority number (RPN).[90] This allows researchers to prioritize experimental efforts based on criticality. Thus, while QbD is primarily concerned with achieving product quality through robust design, risk assessment complements it by systematically evaluating failure points and hazards, ultimately enhancing the efficiency and reliability of the development process.

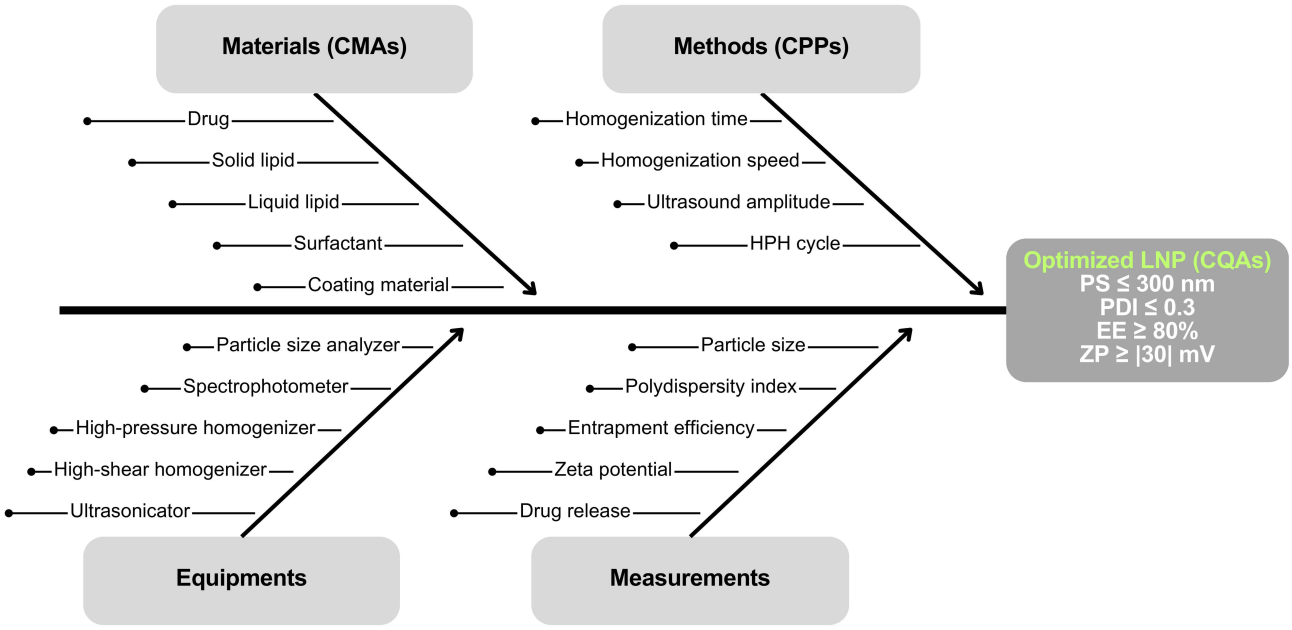

Figure 3 Example of an Ishikawa diagram in LNP optimization.

Design of Experiments

During the QbD course of action, the interactions between independent variables (CMAs and/or CPPs) and dependent variables (CQAs) were formalized through the design of experiments (DoE). In the optimization step, these interactions are typically represented by a polynomial equation that captures single-factor effects, two-factor interactions, and quadratic relationships.[146] The generated polynomial equation describes the interplay between factors, providing recommended paths to follow upscaled production. Various aspects are considered when selecting DoE models, such as the purpose of the investigation, factors and responses to be investigated (number, levels, qualitative or quantitative), resources (materials, time, and budget), prior knowledge, and historical data.[147] Based on the objective of this study, DoE is generally categorized into screening and optimization designs.[148] To concise this article, the DoE models that are commonly used in the production of LNPs are briefly reviewed. More in-depth information regarding the technical details of DoE models has been discussed extensively in several other studies.[149–151]

Screening Designs

A screening design is considered an initial approach to isolate potentially more significant factors from the numerous possible factors influencing responses. Although methodically different, the function of the screening design is similar to that of risk assessment.[152] In general, a screening step is used only to determine the important variables experimentally observed in subsequent optimization designs. Several models, such as two-level full factorial, fractional factorial, Plackett-Burman, and Taguchi, are the most frequently employed screening designs in pharmaceutical formulations.[153]

Factorial design is one of the most comprehensive DoE models which allows multiple factors to be screened simultaneously. The full factorial design (FFD) examines all possible combinations of factor levels, ensuring a comprehensive analysis of the main effects and interactions. A two-level FFD is commonly employed during the screening step, with levels denoted as high (+1) and low (−1) (Figure 4a). The total number of experimental runs is $n = 2^k$, where 2 and k represent the number of levels and factors, respectively.[70] This design allows the evaluation of both the main effects and the interaction effects between the variables. However, as the number of factors increases, the required number of experiments increases exponentially, making it impractical for a large number of factors. To address this, fractional factorial design (FrFD) offers a more efficient alternative by selecting only a subset (fraction) of the full factorial runs while still capturing the significant effects (Figure 4b). Instead of performing all the 2^k experiments, a fraction, such as half (2^{k-1}) or a quarter (2^{k-2}) of the total

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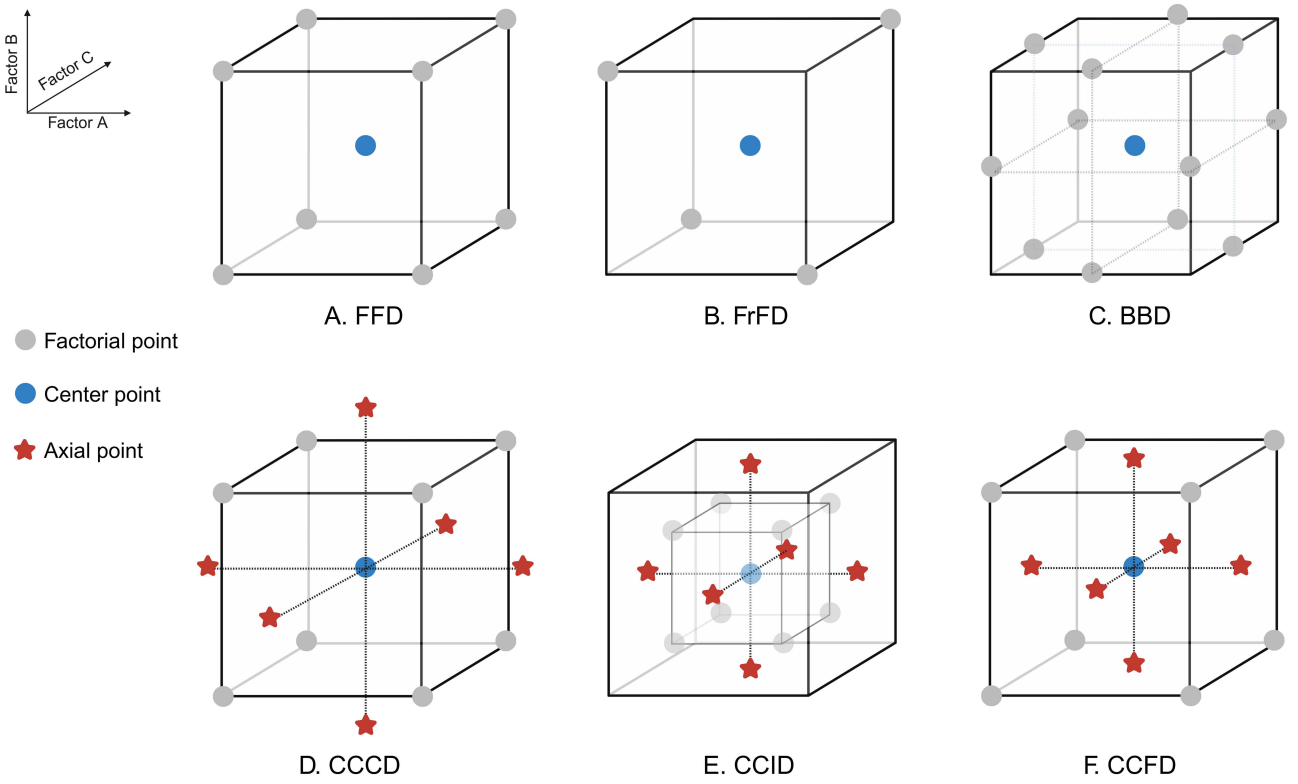

Figure 4 Schematic illustration of Full Factorial Design, FFD (**A**); Fractional Factorial Design, FrFD (**B**); Box-Behnken Design, BBD (**C**); Central Composite Circumscribed Design, CCCD (**D**); Central Composite Inscribed Design, CCID (**E**); Central Composite Face-centered Design, CCFD (**F**). Created in BioRender. Suliman, (K) (2025) https://BioRender.com/10a19ff.

runs, was conducted.[151] For example, in a four-factor scenario, instead of performing all 16 runs for a full 2^4 design, an FrFD with $2^{4-1} = 8$ runs may be sufficient for the initial screening.

The Plackett-Burman design (PBD) is a highly efficient screening design that focuses solely on identifying the most critical factors among a large number of factors while maintaining a minimum number of experiments. Unlike factorial designs, PBD focuses exclusively on estimating the main effects and assumes that interaction effects are negligible.[150] The total number of runs in a PBD follows a multiplication of four greater than the number of factors. For instance, the design may require only twelve runs when there are seven or eight factors to screen.[154,155] This makes PBD particularly useful for preliminary screening, where the goal is to quickly eliminate insignificant factors before moving to a more detailed optimization phase. However, because interaction effects are not accounted for, PBD is best suited for cases in which factor interactions are either minimal or of no primary interest.[71]

The Taguchi design is a specialized factorial design that incorporates orthogonal arrays to systematically reduce the number of experiments while ensuring robust results.[156] However, in contrast to traditional factorial designs that focus on the main effects and interactions, the Taguchi design emphasizes the improvement of process stability using a signal-to-noise (S/N) ratio to quantify the stability and performance of a system under varying conditions. In the experimental setting, the signal (S) represents the desired output quality, whereas the noise (N) represents undesired response variability due to uncontrolled factors or external disturbances.[157] Depending on the desired outcome of the experiment, the S/N ratio can be classified as larger-the-better (LTB), smaller-the-better (STB), or nominal-the-best (NTB).[158] For example, in a study on erythrocyte-coated NLC, coating factors affecting particle size and PDI were screened based on the STB S/N ratio, successfully yielding ultrasmall NLC with potential for glioblastoma therapy.[159] The orthogonal arrays in the Taguchi design allowed for a wide range of experimental possibilities. A two-level design is commonly used to screen for multiple factors in fewer runs. For instance, Pant et al conducted a 7-factor, 2-level experiment with only 8 runs (L_8) using the Taguchi design, instead of 128 (2^7), to identify the critical factors in the production of raloxifene-

loaded NLC.[129] To effectively perform screening using the Taguchi design, it is essential to carefully select experiments that maintain a statistical balance and provide an unbiased estimation of the effect of each factor on the responses.

Optimization Designs

While FFD are often used for screening, they can also be applied in optimization by incorporating three or more levels per factor. A three-level FFD (3^k) introduces an additional intermediate level (coded as 0), allowing for a more precise estimation of the quadratic effects.[147] This is particularly useful when researchers suspect that the relationships between factors and responses are nonlinear. However, similar to two-level FFD, the total number of runs increases exponentially, making this approach efficient only when working with a small number of factors. Notably, a two-level FFD may be applied in an optimization that employs fewer factors.[160] Mendes et al constructed two types of designs to develop NLC containing atorvastatin calcium: 3^2 (nine runs) and 2^2 (four runs) FFD. An observation of the influence of surfactant concentration on the particle size revealed similar interactions in both designs.[159]

Central composite design (CCD) is one of the most extensively used response surface methodology (RSM) designs for optimization because it is effective for modeling curvature and optimizing nonlinear processes. The CCD consists of three main components: a full factorial or fractional factorial cube (2^k), axial (star) points (2k), and center points. The total number of runs required is determined by the formula $2^k + 2k + cp$, where k is the number of factors, and cp is the number of center points. Thus, for a 3-factor optimization, a minimum of 15 experiments were required. The axial points (coded as $+\alpha$ and $-\alpha$) may extend the design space beyond the factorial region, allowing observations at extreme values.[141] Based on the selection of α values, CCD can be categorized as: a) circumscribed (CCCD): the axial points extend beyond the factorial space (Figure 4d); b) inscribed (CCID): the axial points remain within the factorial region (Figure 4e); and c) face-centered (CCFD): the axial points are positioned on the faces of the factorial cube (Figure 4f).[161] The α value in the CCD varies between 1 and $2^{k/4}$, with the latter usually selected to maintain the design rotatability. For example, Ayed et al used a rotatable CCD to optimize two factors, the lipid and surfactant amounts, over 13 runs (including five center points) in the production of quetiapine fumarate-loaded NLC, with an α value of 1.414 ($2^{2/4}$).[131] A similar design was employed in a 3-factor, 20-run optimization of NLC containing ifosfamide (including six center points), with an α value of 1.682 ($2^{3/4}$).[91] It is also noteworthy that the α value in CCD, particularly in face-centered design, can be set at the same level as the low (−1) and high (+1) values of the factorial space, omitting observations at extreme points, as demonstrated in the study of diacerein-loaded SLN performed by Al-Remawi et al.[86]

The Box-Behnken design (BBD) is another widely used optimization design, particularly when the relationship between the factors and responses is expected to be nonlinear. Unlike CCD, which includes axial points that may extend beyond the factorial space, BBD distributes the experimental runs evenly at the midpoint of the factor pairs, eliminating the need to test for extremely high and low values (Figure 4c).[146] CCD, particularly the circumscribed design, includes axial points that extend beyond the factorial region, which can be valuable for exploring a broader response surface but may introduce impractical conditions. On the other hand, face-centered CCD maintains experimental points within the factorial space but requires more runs because of more levels on account of axial points ($+\alpha$ and $-\alpha$).[153] The number of runs in a BBD is given by the formula $2k\ (k-1) + cp$, where k represents the number of factors, and cp represents the number of center points. This means that to optimize three factors, a minimum of 13 experiments are required, whereas CCD requires at least 15 runs. This makes BBD a more efficient choice for optimization in fewer runs, while still maintaining a robust quadratic model. In the development of LNPs, BBD can be used as a follow-up to the screening stage. For instance, in a study of lurasidone HCl-loaded SLN fabricated by high-pressure homogenization, Patel et al screened seven factors using the Plackett-Burman design. Highly critical factors, namely lipid concentration, homogenization pressure, and homogenization cycle, were subsequently optimized to obtain the preferred particle size and entrapment efficiency using a 15-run BBD (including three center points).[112] Nevertheless, numerous studies have reported the direct implementation of BBD for SLN or NLC optimization using 1 − 5 center points, resulting in 13 − 17 experiments.[102,110,113,125]

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Design Space

In developing LNPs, identifying an optimal design space is essential to ensure a well-balanced formulation that meets predefined quality attributes because each factor can influence a response differently, sometimes even in a contradictory manner. For example, increasing the sonication time may reduce the particle size, but it can also lead to an undesirably low entrapment efficiency.[68,103,104,124] Therefore, it is important to describe an optimal space that simultaneously achieves a balanced response for multiple factors. The selection of the most optimized formula is guided by the established DoE results, which help map the relationships between the critical factors and their respective responses. This process involves defining a design space that serves as a multidimensional region where the combination of input variables ensures the achievement of optimal responses.[74] One of the most effective tools in this process is contour plot overlay, which enables the simultaneous evaluation of multiple responses by superimposing their individual contour plots.[162] This graphical approach helps to identify an intersection where all quality attributes meet predefined requirements, thereby defining the most suitable formulation space. In addition, a desirability function is commonly used to simultaneously optimize multiple responses. This method transforms each interaction into a desirability scale ranging from 0 (least desirable) to 1 (most desirable), allowing the calculation of a composite desirability index that reflects the overall optimization outcome.[163] By assigning specific weights to different responses based on their importance, this function aids in resolving conflicting optimization criteria, ensuring that the selected formulation maintains a balance among all critical parameters.

Optimization of Lipid Nanoparticles for Oral Drug Delivery

In QbD-based studies on the development of LNPs for oral administration, various independent variables (CMAs and/or CPPs) have been examined to elucidate their effects on the dependent variables (CQAs). The formation of LNPs relies on physicochemical principles such as lipid melting and recrystallization, emulsification, and colloidal stabilization. Upon cooling, the dispersed lipid phase solidifies into nanoparticles, while surfactants reduce the interfacial tension and provide steric or electrostatic stabilization to maintain colloidal stability.[137] The nature and concentration of lipids, their crystallinity, and the compatibility with surfactants play key roles in controlling particle characteristics and drug incorporation. Process parameters, such as homogenization speed, pressure, and sonication time, directly influence the nucleation and growth of particles. Thus, the relationship between the independent variables and LNP formation mechanisms underpins their influence on CQAs. The most commonly evaluated CQAs during the optimization stage included particle size, polydispersity index, entrapment efficiency, drug loading, zeta potential, and drug release, as presented in Table 2.

Influences of Independent Variables on Particle Size

Particle size is considered to be one of the principal CQAs in LNP development. SLNs and NLCs with smaller particle sizes inherently have larger surface areas, facilitating higher drug dissolution and absorption. A smaller particle size is also preferable because of the varied transport mechanisms during oral administration.[25] Several variables significantly affect the size of the LNPs. In most cases, a higher amount of solid lipids leads to an increase in particle size. At higher solid lipid concentrations, the increased viscosity may resist oil droplet breakdown, resulting in a larger particle size.[114] For example, Diwan et al demonstrated that, with other variables held constant, increasing the solid lipid amount from 50 mg to 300 mg led to a particle size increase from 279.2 nm to 837.6 nm during production of SLN containing cilnidipine.[105] A similar trend is also observed in NLC optimization when the total lipid content or solid-to-liquid lipid (S/L) ratio is considered a CMA, particularly when the solid lipid concentration exceeds that of the liquid lipid, as evident in various reports.[53,115,116,128] However, contradictory interactions have been observed in several studies. For instance, in the study of paliperidone-loaded NLC by Rehman et al, a three-fold increase in total lipid concentration (at an S/L ratio of 70:30) resulted in a particle size reduction from 487.9 nm to 332.6 nm.[117] This finding aligns with the results of Pant et al in the development of raloxifene-loaded NLC, in which smaller particles were obtained at higher solid lipid levels.[129]

The use of higher liquid lipid concentrations has only been investigated in a few studies. Predominantly, an increased amount of liquid lipids is associated with a reduction in the particle size. For instance, in the optimization of eplerenone-loaded NLC, Abd-Elhakeem et al demonstrated that increasing the liquid-to-solid lipid (L/S) ratio from 1:1 to 2:1

Table 2 Design of Experiments in the Optimization of LNPs for Oral Drug Delivery

Drug (s) | LNP Type	Preparation Method	DoE	DoE Results	Performances of Optimized LNP	Ref.
Abiraterone acetate | SLN	Melt emulsification and ultrasonication	Screening: mixture design (4 factors, 15 runs) CCD (13 runs) CMAs: solid lipid concentration, surfactant concentrationCQAs: PS, PDI, EE, ZP, DR	• Screening result: Glyceryl monooleate and polysorbate 80 were selected as solid lipid and surfactant • Relationship between variables: ↓ PS: ↑ surfactant concentration ↑ PS: ↑ lipid concentration ↓ PDI: ↑ surfactant concentration ↑ PDI: ↑ lipid concentration ↑ EE: ↑ lipid concentration, ↑ surfactant concentration↓ ZP: ↑ lipid concentration ↑ ZP: ↑ surfactant concentration ↑ DR: ↑ lipid concentration, ↑ surfactant concentration • Optimized formulation: 4.4% solid lipid, 3.6% surfactant, resulting PS 287.67 nm, PDI 0.138, EE 94%, ZP −25 mV, and DR 98.7%	In vitro drug release study: • ↓ drug release rate (in PBS pH 4.5) • First-order release kinetics model • Fickian drug release Ex vivo gut permeation study: • ↑ cumulative permeated drug In vivo pharmacokinetic study: • ↑ C_{max}, AUC, relative bioavailability • ↓ T_{max}, $t_{1/2}$	[109]
Acyclovir | SLN	High shear homogenization and ultrasonication	CCD (13 runs) CMAs: solid lipid concentration, surfactant concentration CQAs: PS, ZP, PDI	• Relationship between variables: ↓ PS: ↑ surfactant concentration ↑ PS: ↑ lipid concentration ↓ ZP: ↑ surfactant concentration ↑ ZP: ↑ lipid concentration ↓ PDI: ↑ lipid concentration, ↑ surfactant concentration • Optimized SLN resulting PS 104.89 nm, ZP −37 mV, and PDI 0.21. However, the optimal variables to obtain them were not explained.	In vitro drug release study: • ↓ drug release rate (in SGF pH 1.2) • ↓ drug release rate (in SIF pH 6.8)In vivo pharmacokinetic study: • ↑ C_{max}, AUC, $t_{1/2}$, relative bioavailability • ↔ T_{max} • ↓ K_{el}	[106]
Alendronate sodium | SLN	Melt emulsification and ultrasonication	BBD (15 runs) CMAs: solid lipid concentration, surfactant concentration CPPs: sonication time CQAs: PS, EE	• Relationship between variables:↓ PS: ↑ sonication time↑ PS: ↑ lipid concentration, ↑ surfactant concentration↓ EE: ↑ surfactant concentration, ↑ sonication time ↑ EE: ↑ lipid concentration • Optimized formulation: 30% w/w solid lipid, 5% w/w surfactant, 8 min sonication, resulting PS 98 nm and EE 74.3%	In vitro drug release study: • ↓ cumulative drug release (in 0.1 N HCl pH 1.2), compared to commercial Fosamax® tablet • In PBS pH 7.4 (not compared) the release of Alendronate sodium from SLN showed sustained release In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, AUC, $t_{1/2}$, relative bioavailability • ↓ K_{el}	[110]
Amisulpride | NLC	Solvent evaporation	FFD (24 runs) CMAs: type of solid lipid, lipid/drug ratio, type of external suspending medium CQAs: PS, EE	• Relationship between variables: ↓ PS: lower in gelucire 43/1, lower in 0.5% TSP medium ↑ PS: ↑ lipid/drug ratio↑ EE: higher in gelucire 43/1, ↑ lipid/drug ratio, higher in 1% HPMC medium • Selected formulation: gelucire 43/1 as solid lipid, 13:1 lipid/drug, and 1% HPMC as external suspending medium, resulting smaller PS and higher EE	In vitro drug release study: • Rapid release in 0.1 N HCl pH 1.2 for 2h, followed by slower release in PBS pH 7.4 for the next 6 h In vivo pharmacokinetic study: • ↑ C_{max}, AUC, $t_{1/2}$, relative bioavailability • ↓ K_{el}	[133]

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Table 2 (Continued).

Drug (s) | LNP Type	Preparation Method	DoE	DoE Results	Performances of Optimized LNP	Ref.
Apigenin | SLN	Melt emulsification and ultrasonication	BBD (15 runs) CMAs: solid lipid concentration, surfactant concentration CPPs: sonication time CQAs: PS, EE	• Relationship between variables:↓ PS: ↑ surfactant concentration, ↑ sonication time ↑ PS: ↑ lipid concentration ↓ EE: ↑ sonication time↑ EE: ↑ lipid concentration, ↑ surfactant concentration • Optimized formulation: 320 mg solid lipid, 220 mg surfactant, 5 min sonication, resulting PS 161.7 nm and EE 80.44%	In vitro drug release study: • ↑ cumulative drug release (in PBS pH 7) Ex vivo gut permeation study: • ↑ permeation flux Antioxidant study: • ↑ antioxidant activity Anti-arthritic study: • ↓ TNF-α, IL-1β, paw edema	[114]
Apixaban | NLC	High pressure homogenization	FFD (8 runs) CMAs: surfactant concentration CPPs: HPH pressure, HPH cycle CQAs: PS, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ HPH pressure, ↑ HPH cycle ↓ EE: ↑ HPH pressure, ↑ HPH cycle EE: ↑ surfactant concentration • Optimized formulation: 1% surfactant, 800 bar HPH pressure, 9 HPH cycles, resulting PS 232 nm and EE 91.9%	In vitro drug release study: • ↓ cumulative drug release (in PBS pH 6.8) • Higuchi release kinetics model In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, AUC, $t_{1/2}$	[111]
Atazanavir | NLC	High pressure homogenization	CCRD (20 runs)CMAs: total lipid concentration, surfactant concentrationCPPs: HPH pressureCQAs: PS, PDI	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ HPH pressure ↑ PS: ↑ lipid concentration ↓ PDI: ↑ surfactant concentration ↑ PDI: ↑ lipid concentration, ↑ HPH pressure • Optimized formulation: 1.89% total lipid, 1.93% surfactant, 779.96 bar HPH pressure, resulting PS 89.02 nm and PDI 0.222	In vitro drug release study: • ↑ cumulative drug release (in SGF pH 1.2 for 2h, continued in SIF pH 6.8 for 22 h) • Korsmeyer-Peppas release kinetics model • Non-Fickian drug release In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, AUC, MRT, relative bioavailability • Chylomicron flow block model indicates lymphatic uptake of NLC	[64]
Azilsartan | NLC	High pressure homogenization	FFD (17 runs) CMAs: total lipid concentration, surfactant concentration CPPs: HPH cycles CQAs: PS, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ HPH cycles ↑ PS: ↑ lipid concentration ↓ EE: ↑ surfactant concentration, ↑ HPH cycles ↑ EE: ↑ lipid concentration • Optimized formulation: 1 g total lipid, 0.5 g surfactant, 10 HPH cycles, resulting PS 274.2 nm and EE 88%	In vitro drug release study: • ↓ drug release rate (in PBS pH 6.8) • Higuchi release kinetics model • Non-Fickian drug release In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, AUC, $t_{1/2}$, MRT • ↓ K_{el}	[116]
Bergenin | NLC	Melt emulsification and ultrasonication	BBD (13 runs) CMAs: total lipid concentration, surfactant concentration CPPs: sonication time CQAs: PS, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ sonication time ↑ PS: ↑ lipid concentration ↓ EE: ↑ surfactant concentration ↑ EE: ↑ lipid concentration, ↑ sonication time • Optimized formulation: 3.5% total lipid, 3% surfactant, 7 min sonication, resulting PS 174.3 nm and EE 80.6%	In vitro drug release study: • ↑ cumulative drug release (in PBS pH 6.8) • Korsmeyer-Peppas release kinetics model • Non-Fickian drug release Ex vivo gut permeation study: • ↑ apparent permeability coefficient In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, AUC, $t_{1/2}$ • ↓ K_{el} In vivo anti-inflammatory activity: • Bergenin-loaded NLC showed slower but prolonged effect compared to free drug	[102]

Carvedilol | SLN	High shear homogenization and ultrasonication	BBD (15 runs) CMAs: solid lipid concentration, surfactant concentration, stearylamine (positive charge inducing agent) concentration CQAs: PS, ZP, EE, DR, T85%	• Relationship between variables: ↓ PS: ↑ surfactant concentration ↑ PS: ↑ lipid concentration, ↑ stearylamine concentration ↓ ZP: ↑ surfactant concentration ↑ ZP: ↑ lipid concentration, ↑ stearylamine concentration ↑ EE: ↑ lipid concentration, ↑ surfactant concentration, ↑ stearylamine concentration ↓ DR: ↑ lipid concentration, ↑ stearylamine concentration ↑ DR: ↑ surfactant concentration ↓ T85%: ↑ surfactant concentration, ↑ T85%: ↑ lipid concentration, ↑ stearylamine concentration • Optimized formulation: 11.9% solid lipid, 2.87% surfactant, 6% stearylamine, resulting PS 31.3 nm, ZP 24.25 mV, EE 91.43%, DR 17.55%, and T85% 8.92 h	In vitro drug release study: • Controlled release behavior was found in all formulations (not compared to free drug) • Hixson-Crowell release kinetics model • Non-Fickian drug release • In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, $t_{1/2}$, AUC, MRT, relative bioavailability • ↓ clearance, K_{el}	[123]
Cilnidipine | SLN	Emulsification and solvent evaporation	Screening: mini run resolution IV design (5 factors, 12 runs) BBD (17 runs) CMAs: solid lipid concentration, internal to external phase ratio CPPs: temperature CQAs: PS, DL	• Screening result: solid lipid concentration, internal to external phase ratio, and temperature were the most influential factors. • Relationship between variables (at given temperatures: 50, 70, 90 ° C): ↓ PS: ↑ phase ratio ↑ PS: ↑ lipid concentration ↓ DL: ↑ lipid concentration ↑ DL: ↑ phase ratio • Optimized formulation: 57.03 mg solid lipid, 0.14 internal to external phase ratio, 84 °C temperature, resulting PS 207.1 nm and DL 15.9%	In vitro drug release study: • ↓ cumulative drug release (in PBS pH 7.4) • Makoid-Banakar release kinetics model In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, AUC, MRT, relative bioavailability • ↓ clearance In vivo pharmacodynamic study: • NLC system showed higher and more sustained hypertensive effect	[105]
Dapagliflozin | NLC	High pressure homogenization	BBD (17 runs) CMAs: S/L lipid ratio, surfactant concentration CPPs: HPH cycles CQAs: PS, EE, DR	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ HPH cycles ↑ PS: ↑ lipid ratio↑ EE: ↑ lipid ratio, ↑ surfactant concentration, ↑ HPH cycles ↓ DR: ↑ lipid ratio, ↑ DR: ↑ surfactant concentration, ↑ HPH cycles • Optimized formulation: 8:3 solid/liquid lipid, 3% surfactant, 4 HPH cycles resulting PS 231.9 nm, EE 88.32%, and DR 78.58%	In vitro drug release study: • ↑ cumulative drug release in (SIF pH 6.8) • Korsmeyer-Peppas release kinetics model • Non-Fickian drug release Ex vivo gut permeation study: • ↑ apparent permeability coefficient	[115]
Dapagliflozin | SLN	High shear homogenization and ultrasonication	BBD (17 runs)CMAs: solid lipid concentration, surfactant concentrationCPPs: homogenization timeCQAs: DR, EE, PS	• Relationship between variables: ↓ DR: ↑ surfactant concentration ↑ DR: ↑ lipid concentration, ↑ homogenization time ↓ EE: ↑ lipid concentration, ↑ homogenization time ↑ EE: ↑ surfactant concentration ↓ PS: ↑ surfactant concentration, ↑ homogenization time ↑ PS: ↑ lipid concentration • Optimized formulation: 1% w/v solid lipid, 20% w/v surfactant, 2 min homogenization time, resulting PS 100.13 nm, EE 94.46%, and DR 99.08%	In vitro drug release study: • ↑ cumulative drug release (in 0.1 N HCl pH 1.2 for 2h, continued in PBS pH 7.4 for 22 h) • Korsmeyer-Peppas release kinetics model In vivo pharmacokinetic study: • ↑ C_{max}, AUC, K_{el} • ↔ T_{max} • ↓ $t_{1/2}$In vivo anti-diabetic study: • ↓ blood glucose level, glycosylated hemoglobin • ↑ insulin level • Biochemical study: • ↓ triglycerides, total cholesterol, LDL, uric acid, urea, SGOT, SGPT, ALP • ↑ HDL	[108]

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Table 2 (Continued).

Drug (s) | LNP Type	Preparation Method	DoE	DoE Results	Performances of Optimized LNP	Ref.
Diacerein | SLN	High shear homogenization	CCD (13 runs)CMAs: solid lipid concentration, surfactant concentrationCQAs: PS, ZP, unpleasant taste	• Relationship between variables: ↔ PS ↓ ZP: ↑ lipid concentration, ↑ surfactant concentration↑ unpleasant taste: ↑ lipid concentration, ↑ surfactant concentration • Optimized formulation: 2% solid lipid, 0.9% surfactant, resulting PS 48.58 nm, ZP −18.65 mV, and unpleasant taste 39.4%	In vitro drug release study: • No release in 0.1 M HCl • Rapid release in PBS pH 6.8 + 2% SLS • Release profiles of Diacerein SLN stored in amber glass and in sachet were intact after 6 months storage In vivo anti-diarrheal study: • ↓ defecation output, frequency of defecation	[86]
Doxorubicin | NLC	Melt emulsification and ultrasonication	CCD (10 runs) CMAs: surfactant concentration, pH CQAs: PS, EE	• In the formulation, cetyl palmitate (PAL) and Gelucire® 43/01 (GEL) was used as solid lipids in different NLC-DOX systems • Relationship between variables: ↔ PS (GEL-DOX) ↓ PS (PAL-DOX): ↑ surfactant concentration↑ PS (PAL-DOX): ↑ pH ↓ EE (GEL-DOX): ↑ pH, ↑ surfactant concentration ↓ EE (PAL-DOX): ↑ pH ↑ EE (PAL-DOX): ↑ surfactant concentration • NLC GEL-DOX in pH 1 with 85 mg surfactant resulting smaller size compared to NLC PAL-DOX in the same condition. However, the latter resulting higher EE, which is more critical than smaller size.	In vitro drug release study: • All DOX-loaded NLC formulations showed lower release in regime I (FaSSGF pH 1.6) and II (FaSSIF pH 6.5) and higher release in regime III (PBS pH 7.4) and IV (citrate buffer solution pH 6.3) • Korsmeyer-Peppas release kinetics model • Fickian (regime II and III), non-Fickian (regime IV), super case II (regime I) In vitro cytotoxicity study: • All DOX-loaded NLC formulations showed enhanced toxicity against MDA-MB-231 cell, close to that obtained from free DOX • Functionalized NLC (with PEG-FA) increased cyto-toxicity In vitro cell uptake study: • DOX-loaded gelucire NLC showed faster cell uptake compared to cetyl palmitate NLC • Functionalized NLC (with PEG-FA) increased cell uptake	[49]
Efavirenz | NLC	Phase inversion temperature	BBD (17 runs) CMAs: oil phase concentration, surfactant concentration, volume ratio of diluting aqueous phase to initial emulsionCQAs: PS, PDI, ZP, EE, DL, DR	• Relationship between variables: ↓ PS: ↑ surfactant concentration ↑ PS: ↑ oil phase concentration ↓ PDI: ↑ surfactant concentration ↑ PDI: ↑ oil phase concentration ↓ ZP: ↑ oil phase concentration, ↑ volume ratio ↑ ZP: ↑ surfactant concentration ↓ EE: ↑ oil phase concentration ↑ EE: ↑ surfactant concentration, ↑ volume ratio ↓ DL: ↑ oil phase concentration, ↑ surfactant concentration↑ DL: ↑ volume ratio ↓ DR: ↑ surfactant concentration ↑ DR: ↑ oil phase concentration, ↑ volume ratio • Optimized formulation: 17.5% oil phase, 10% surfactant, 3.5 of diluting aqueous phase to initial emulsion ratio, resulting PS 60.71 nm, PDI 0.09, ZP −35.93 mV, EE 92.6%, DL 7.39%, and DR 55.96%	In vitro drug release study: • Higuchi release kinetics model • Non-Fickian drug release • In vitro cytotoxicity study: • ↔ Caco-2 cell viability • Ex vivo gut permeation study: • ↑ cumulative permeated drug, apparent permeability coefficient	[130]

Efavirenz | NLC	High pressure homogenization	CCRD (14 runs) CMAs: total lipid concentration, surfactant concentration CPPs: HPH pressure CQAs: PS, PDI, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ HPH pressure ↑ PS: ↑ lipid concentration ↓ PDI: ↑ surfactant concentration, ↑ HPH pressure ↑ PDI: ↑ lipid concentration ↑ EE: ↑ lipid concentration, ↑ surfactant concentration, ↑ HPH pressure • Optimized formulation: 1.5% total lipid, 2% surfactant, 750 bar HPH pressure, resulting PS 106 nm, PDI 0.235, and EE 92%	In vitro drug release study: • ↑ cumulative drug release (in 0.1 N HCl pH 1.2 for 2h, continued in PBS pH 6.8 for 22 h) • Higuchi release kinetics model • Non-Fickian drug release In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, AUC, MRT, relative bioavailability Toxicity study: • ↔ SGOT, SGPT, ALP (in low and medium doses, after 28 days) • ↑ SGOT, SGPT, ALP (in high dose, after 28 days)	[128]
Entacapone | NLC	Melt emulsification and ultrasonication	FFD (8 runs) CMAs: total lipid concentration, surfactant concentration CPPs: sonication time CQAs: PS, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ sonication time ↑ PS: ↑ lipid concentration ↓ EE: ↑ sonication time↑ EE: ↑ lipid concentration, ↑ surfactant concentration • Optimized formulation: 0.5% total lipid, 1–2% surfactant, 13–15 min sonication, resulting PS 161.2 nm and EE 82.5%	In vitro drug release study: • ↓ drug release rate (in 0.1 N HCl pH 1.2 for 2h, continued in PBS pH 6.8 for 22 h) • Korsmeyer-Peppas release kinetics model • Non-Fickian drug release In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, AUC, $t_{1/2}$, MRT • ↓ K_{el}	[121]
Eplerenone | NLC	Melt emulsification and ultrasonication	D-optimal (19 runs) CMAs: L/S lipid ratio, surfactant concentration, type of surfactant CQAs: PS, PDI, ZP, EE	• Relationship between variables: ↓ PS: ↑ lipid ratio, lower in Pluronic F127 ↑ PS: ↑ surfactant concentration ↓ PDI: ↑ lipid ratio ↑ PDI: ↑ surfactant concentration ↓ EE: ↑ lipid ratio, ↑ surfactant concentration↑ EE: higher in Pluronic F127 ↔ ZP • Optimized formulation: 2:1 liquid/solid lipid, 0.45% Pluronic F127, 45s sonication, resulting PS 134 nm, PDI 0.31, EE 76%, and ZP −32.37 mV	In vitro drug release study: • ↑ cumulative drug release (in PBS pH 7.4) Ex vivo gut permeation study: • ↑ cumulative permeated drug	[118]
Etravirine and Darunavir Ethanolate | NLC	Melt emulsification and ultrasonication	CCRD (20 runs) CMAs: binary mixture (BM) concentration, surfactant concentration CPPs: sonication time CQAs: PS, PDI, EE (ETR), EE (DRVE)	• Relationship between variables: ↓ PS: ↑ surfactant concentration, sonication time ↑ PS: ↑ BM concentration ↓ PDI: ↑ surfactant concentration, sonication time ↑ PDI: ↑ BM concentration ↓ EE (ETR): ↑ surfactant concentration, ↑ sonication time ↑ EE (ETR): ↑ BM concentration ↓ EE (DRVE): ↑ surfactant concentration, ↑ sonication time ↑ EE (DRVE): ↑ BM concentration • Optimized formulation: 4% w/w BM, 3% w/w surfactant, 120 s sonication, resulting PS 171.23 nm, PDI 0.135, EE (ETR) 96.56%, and EE (DRVE) 94.22%	In vitro drug release study: • ↑ cumulative drug release (in 0.1 N HCl pH 1.2) • ↑ cumulative drug release (in acetate buffer pH 4.5) • ↑ cumulative drug release (in PBS pH 6.8) • Korsmeyer-Peppas release kinetics model Ex vivo everted gut sac study: • ↑ apparent permeability coefficient In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, AUC, $t_{1/2,}$ MRT, bioavailability • Chylomicron flow block model indicates lymphatic uptake of NLC Anti-HIV-1 study: • NLCs containing ETR, DRVE and ETR-DRVE showed higher anti-HIV-1 activity compared to pure drugs	[124]

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Table 2 (Continued).

Drug (s) | LNP Type	Preparation Method	DoE	DoE Results	Performances of Optimized LNP	Ref.
Exemestane and Thymoquinone | NLC	Melt emulsification and ultrasonication	BBD (17 runs) CMAs: solid lipid concentration, surfactant concentration CPPs: sonication time CQAs: PS, PDI, EE (EXE), EE (THY)	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ sonication time ↑ PS: ↑ solid lipid concentration ↓ PDI: ↑ surfactant concentration, ↑ sonication time ↑ PDI: ↑ solid lipid concentration ↓ EE (EXE): ↑ surfactant concentration, ↑ sonication time ↑ EE (EXE): ↑ solid lipid concentration ↓ EE (THY): ↑ surfactant concentration, ↑ sonication time ↑ EE (THY): ↑ solid lipid concentration • Optimized formulation: 350 mg solid lipid, 200 mg surfactant, 4 min sonication, resulting PS 268.2 nm, PDI 0.155, EE (EXE) 76.2%, and EE (THY) 75.1%	In vitro drug release study: • ↓ cumulative drug release (in SGF pH 1.2) • ↑ cumulative drug release (in SIF pH 6.8) • Korsmeyer-Peppas release kinetics model • Fickian drug release Ex vivo gut permeation study: • ↑ cumulative permeated drug, apparent permeability coefficient In vitro cell uptake study: • ↑ MCF-7 cell uptake In vitro cytotoxicity study: • ↑ MCF-7 cell cytotoxicity	[103]
Ezetimibe | NLC	High pressure homogenization	FFD (8 runs) CMAs: surfactant concentration CPPs: HPH pressure, HPH cycles CQAs: PS, EE	• Relationship between variables: ↓ PS: ↑ HPH pressure, ↑ HPH cycles ↑ PS: ↑ surfactant concentration ↓ EE: ↑ surfactant concentration ↑ EE: ↑ HPH pressure, ↑ HPH cycles • Optimized formulation: 0.5% surfactant, 700 bar HPH pressure, 7 HPH cycles, resulting PS 134.5 nm and EE 91.32%	In vitro drug release study: • Sustained drug release (in acetate buffer pH 4.5) • Higuchi release kinetics model • Non-Fickian drug release In vivo anti-hyperlipidemic study: • ↓ triglycerides, total cholesterol, LDL • ↑ HDL	[120]
Fenofibrate | SLN	Hot-melt extrusion	Plackett-Burman design (8 factors, 12 runs) CMAs: drug concentration, solid lipid concentration, surfactant concentration, type of lipid, type of surfactant CPPs: screw speed, barrel temperature, zone of liquid addition CQAs: PS, PDI, ZP, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ barrel temperature, lower in Compritol® 888 ATO, lower in Cremophor® EL ↑ PS: ↑ drug concentration, ↑ lipid concentration ↓ PDI: ↑ surfactant concentration, lower in Compritol® 888 ATO ↑ PDI: ↑ lipid concentration ↓ ZP: ↑ lipid concentration ↑ ZP: ↑ drug concentration, ↑ surfactant concentration ↓ EE: ↑ zone of addition ↑ EE: ↑ drug concentration, ↑ lipid concentration, ↑ screw speed, higher in Compritol® 888 ATO, higher in Cremophor® EL • Optimized formulation: 0.5% w/w drug, 8% w/w Compritol® 888 ATO, 3% w/w Cremophor® EL, 240 rpm screw speed, 150 °C barrel temperature, and zone 4 as zone of liquid addition, resulting PS 125 nm, PDI 0.284, ZP 39.28 mV, and EE 78.46%	In vitro drug release study: • ↑ cumulative drug release rate (in PBS pH 7.4) In vivo pharmacokinetic study: • ↑ C_{max}, AUC • ↓ T_{max}	[56]
Fexofenadine HCl | NLC	Solvent injection	FFD (9 runs) CMAs: S/L lipid ratio, surfactant concentration CQAs: PS, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration ↑ PS: ↑ S/L lipid ratio ↓ EE: ↑ S/L lipid ratio, ↑ surfactant concentration • Optimized formulation: 6:4 solid/liquid lipid, 0.5% w/v surfactant, resulting PS 127.5 nm and EE 81.31%	In vitro drug release study: • ↓ cumulative drug release (in PBS pH 1.2 for 2h, continued in PBS pH 6.8 for 48 h) • Higuchi release kinetics model • Fickian drug release In vivo pharmacokinetic study: • ↑ C_{max}, AUC, $t_{1/2}$, relative bioavailability • ↓ T_{max}	[53]

Fexofenadine HCl | SLN	High shear homogenization and ultrasonication	FFD (9 runs) CMAs: surfactant concentration, polymer molecular weight CQAs: PS, ZP, PDI, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration ↑ PS: ↑ polymer MW ↑ ZP: ↑ polymer MW ↓ PDI: ↑ surfactant concentration, ↑ polymer MW ↓ EE: ↑ surfactant concentration ↑ EE: ↑ polymer MW • Optimized formulation: 200 mg surfactant, low MW polymer, resulting PS 229 nm, ZP 36.3 mV, PDI 0.23, and EE 64.9%	In vitro drug release study: • ↓ cumulative drug release rate (in 0.001 M HCl pH 1.2, PBS pH 6.8, and PBS pH 7.4) • Higuchi release kinetics model In vivo anti-ulcerative colitis study: • ↓ phosphatidylinositol-3 kinase, protein kinase B, TNF-α, IL-6	[48]
Flutamide | NLC	Melt emulsification and ultrasonication	BBD (17 runs) CMAs: total lipid concentration, surfactant concentration CPPs: sonication time CQAs: PS, PDI, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ sonication time ↑ PS: ↑ lipid concentration ↓ PDI: ↑ surfactant concentration ↑ PDI: ↑ lipid concentration, ↑ sonication time ↓ EE: ↑ surfactant concentration, ↑ sonication time ↑ EE: ↑ lipid concentration • Optimized formulation: 3% w/w total lipid, 2.5% surfactant, 4 min sonication, resulting PS 27.66 nm, PDI 0.175, and EE 97.81%	In vitro drug release study: • ↑ cumulative drug release (in 1% SLS) • Korsmeyer-Peppas release kinetics model • Fickian drug release In vitro cytotoxicity study: • ↓ PC3 cell viability In vitro wound healing study: • ↓ cell migration	[127]
Ibrutinib | NLC	Melt emulsification and ultrasonication	Screening: Plackett-Burman design (6 factors, 12 runs) CCD (19 runs) CMAs: liquid lipid concentration, drug concentration, surfactant, concentration CQAs: PS, PDI, EE	• Screening result: liquid lipid concentration, drug concentration, and surfactant concentration were the most influential factors. • Relationship between variables: ↓ PS: ↑ liquid lipid concentration, ↑ PS: ↑ drug concentration, ↑ surfactant concentration↑ PDI: ↑ liquid lipid concentration, ↑ drug concentration EE: ↑ liquid lipid concentration, ↑ surfactant concentration • Optimized formulation: 0.339% liquid lipid, 10% drug, 1.421% surfactant, resulting PS 106.63 nm, PDI 0.283, and EE 74.32%	In vitro drug release study: • ↓ cumulative drug release (in PBS pH 6.8 for 60 h) • Higuchi release kinetics model In vivo pharmacokinetic study: • ↑ C_{max}, $t_{1/2}$, AUC, MRT • Chylomicron flow block model indicates lymphatic uptake of NLC	[119]
Ifosfamide | NLC	Solvent diffusion	CCRD (20 runs) CMAs: drug/lipid ratio, organic/aqueous ratio, surfactant concentration CQAs: PS, DL, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration ↑ PS: ↑ drug/lipid ratio, ↑ organic/aqueous ratio ↓ DL: ↑ organic/aqueous ratio, ↑ surfactant concentration ↑ DL: ↑ drug/lipid ratio ↓ EE: ↑ drug/lipid ratio, ↑ organic/aqueous ratio ↑ EE: ↑ surfactant concentration • Optimized formulation: 1:3 drug/lipid, 1:10 organic/aqueous phase, 1% w/v surfactant, resulting PS 223 nm, DL 6.14%, and EE 77%	In vitro drug release study: • ↓ cumulative drug release in (PBS pH 7.4) • In PBS pH 1.2 and 6.8 (not compared to free drug) the release of ifosfamide from NLC showed initial burst, then plateaued at 13–15% at 6h	[91]
Linagliptin | SLN	Solvent injection and homogenization	CCD (35 runs) CMAs: solid lipid concentration, polymer concentration, surfactant concentration CPPs: homogenization speed CQAs: PS, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ homogenization speed ↑ PS: ↑ lipid concentration, ↑ polymer concentration ↓ EE: ↑ surfactant concentration, ↑ homogenization speed ↑ EE: ↑ lipid concentration, ↑ polymer concentration • Optimized formulation: 84 mg solid lipid, 30.58 mg polymer, resulting PS 332 nm and EE 59.63%	In vitro drug release study: • cumulative drug release in SIF pH 6.8 was significantly higher than in SGF pH 1.2 • compared to free drug, SLN showed relatively slower release (in PBS pH 6.8) • Zero-order release kinetics model In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, AUC, $t_{1/2}$, relative bioavailability • ↓ K_{el}	[46]

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Table 2 (Continued).

Drug (s) | LNP Type	Preparation Method	DoE	DoE Results	Performances of Optimized LNP	Ref.
Lurasidone HCl | SLN	High pressure homogenization	Screening: Plackett-Burman design (7 factors, 8 runs) BBD (17 runs) CMAs: solid lipid concentration CPPs: HPH pressure, HPH cycle CQAs: PS, EE	• Screening result: solid lipid concentration, HPH pressure, and HPH cycle were the most influential factors. • Relationship between variables: ↓ PS: ↑ HPH pressure, ↑ HPH cycle ↑ PS: ↑ lipid concentration ↓ EE: ↑ HPH pressure, ↑ HPH cycle ↑ EE: ↑ lipid concentration • Optimized formulation: 10% w/v solid lipid, 600 bar HPH pressure, 7 HPH cycles, resulting PS 139.8 nm and EE 79.1%	In vitro drug release study: • ↓ drug release rate (in 0.1 N HCl pH 1.2 for 2h, continued in PBS pH 6.8 for 22 h) • Controlled drug release • Higuchi release kinetics model • Non-Fickian drug release Ex vivo drug diffusion study: • ↓ drug diffusion rate • Sustained drug diffusion In vitro cytotoxicity study: • ↔ Caco-2 cell viability In vitro cellular permeability study: • ↑ apparent permeability coefficient In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, AUC, relative bioavailability In vivo pharmacodynamic study: • ↓ escape latency time in pole climbing test • ↓ extrapyramidal side effects in catalepsy test	[112]
Lycopene | NLC	Melt emulsification and ultrasonication	BBD (17 runs) CMAs: solid lipid concentration, surfactant concentration CPPs: sonication time CQAs: PS, PDI, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ sonication time ↑ PS: ↑ lipid concentration ↓ PDI: ↑ surfactant concentration, ↑ sonication time ↑ PDI: ↑ lipid concentration ↓ EE: ↑ sonication time ↑ EE: ↑ lipid concentration, ↑ surfactant concentration • Optimized formulation: 12% w/v solid lipid, 5% surfactant, 45s sonication, resulting PS 121.9 nm, PDI 0.37, and EE 84.5%	In vitro drug release study: • ↓ cumulative drug release (in PBS pH 7.4) • Higuchi release kinetics model • Fickian drug release In vitro antioxidant study: • ↓ IC_{50} Ex vivo gut permeation study: • ↑ cumulative permeated drug, apparent permeability coefficient In vitro cytotoxicity study: • ↓ MDA-MB-231 cell viability	[126]
Nabumetone | NLC	Melt emulsification and ultrasonication	BBD (17 runs) CMAs: total lipid concentration, surfactant concentration CPPs: sonication time CQAs: PS, PDI, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ sonication time ↑ PS: ↑ lipid concentration ↑ PDI: ↑ lipid concentration, ↑ surfactant concentration, ↑ sonication time ↓ EE: ↑ surfactant concentration, ↑ sonication time ↑ EE: ↑ lipid concentration • Optimized formulation: 2.34% total lipid, 2% surfactant, 6.21 min sonication, resulting PS 127 nm, PDI 0.279, and EE 84.4%	In vitro drug release study: • ↑ cumulative drug release in (PBS pH 6.8) • Korsmeyer-Peppas release kinetics model • Fickian drug release In vivo anti-inflammatory activity: • Nabumetone-loaded NLC showed higher inhibition and prolonged effect compared to free drug	[113]

Naringin | NLC	Melt emulsification and ultrasonication	BBD (17 runs) CMAs: total lipid concentration, surfactant concentration CPPs: sonication time CQAs: PS, PDI, EE, DL	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ sonication time ↑ PS: ↑ lipid concentration ↓ PDI: ↑ surfactant concentration ↑ PDI: ↑ lipid concentration, ↑ sonication time ↓ EE: ↑ surfactant concentration, ↑ sonication time ↑ EE: ↑ lipid concentration ↓ DL: ↑ lipid concentration, ↑ surfactant concentration, ↑ sonication time • Optimized formulation: 121 mg total lipid, 96 mg surfactant, 2.46 min sonication, resulting PS 94.45 nm, PDI 0.23, EE 85.33%, and DL 10.04%	In vitro drug release study: • ↑ cumulative drug release (in SGF pH 1.2 for 2h, continued in SIF pH 6.8 for 22 h) • First-order release kinetics model In vitro antioxidant activity: • Slightly increasing antioxidant activity Ex vivo gut permeation study: • ↑ cumulative permeated drug, apparent permeability coefficient Ex vivo intestinal uptake study: • ↑ permeation	[104]
Olmesartan medoxomil | NLC	Melt emulsification and ultrasonication	FCCD (13 runs) CMAs: solid lipid concentration, surfactant concentration CQAs: PS, ZP, EE, DR	• Relationship between variables: ↓ PS: ↑ lipid concentration ↑ PS: ↑ surfactant concentration ↓ ZP: ↑ surfactant concentration ↓ EE: ↑ lipid concentration ↑ DR: ↑ surfactant concentration • Optimized formulation: based on coded levels, the optimized system was obtained at 0.3 solid/liquid lipid level (7:3 solid/liquid lipid) and 0.16 surfactant level (5.2% surfactant), resulting PS 245.5 nm, ZP −19.4 mV, EE 74.7%, and DR 84%	In vitro drug release study: • ↑ cumulative drug release (in SGF pH 1.2 for 2h, continued in SIF pH 6.8 for 10 h) • Korsmeyer-Peppas release kinetics model • Fickian drug release	[132]
Paliperidone | NLC	High pressure homogenization | melt emulsification and ultrasonication	BBD (29 runs) | BBD (17 runs) CMAs: total lipid concentration, surfactant concentration CPPs: HPH pressure, HPH cycle | sonication timeCQAs: PS, EE, DL	• Relationship between variables: ↓ PS: ↑ lipid concentration, ↑ HPH pressure, ↑ HPH cycle, ↑ sonication time ↑ PS: ↑ surfactant concentration ↓ EE: ↑ surfactant concentration ↑ EE: ↑ lipid concentration, ↑ HPH pressure, ↑ HPH cycle, ↑ sonication time ↓ DL: ↑ surfactant concentration ↑ DL: ↑ lipid concentration, ↑ HPH pressure, ↑ HPH cycle, ↑ sonication time • Optimized formulation (HPH): 2.98% total lipid, 3.79% surfactant, 945.72 bar HPH pressure, 4.93 HPH cycles, resulting PS 297.33 nm, EE 78.31%, and DL 7.44%• Optimized formulation (melt emulsification-ultrasonication): 3% total lipid, 5% surfactant, 11.05 min sonication, resulting PS 86.35 nm, EE 90.31%, and DL 8.49%	In vitro drug release study: • ↑ cumulative drug release (in 0.1 N HCl pH 1.2) • ↑ cumulative drug release (in PBS pH 6.8) • ↑ cumulative drug release (in PBS pH 7.4) • Korsmeyer-Peppas release kinetics model • Fickian drug release Ex vivo gut permeation study: • ↑ cumulative permeated drug In vitro lipolysis study: • ↑ drug release in aqueous phase and lipid phase	[117]

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Table 2 (Continued).

Drug (s) | LNP Type	Preparation Method	DoE	DoE Results	Performances of Optimized LNP	Ref.
Pioglitazone | SLN	High shear homogenization	BBD (17 runs) CMAs: solid lipid concentration, surfactant concentration CPPs: homogenization speed CQAs: PS, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ homogenization speed ↑ PS: ↑ lipid concentration ↓ EE: ↑ surfactant concentration, ↑ homogenization speed ↑ EE: ↑ lipid concentration • Optimized formulation: 4.5% w/v solid lipid, 3% w/v surfactant, 3800 rpm homogenization speed, resulting PS 180.65 nm and EE 85.34%	In vitro drug release study: • ↑ cumulative drug release (in 0.1 N HCl pH 1.2 for 2h, continued in PBS pH 7.4 for 22 h) • Korsmeyer-Peppas release kinetics model In vivo anti-diabetic study: • ↓ blood glucose level Biochemical study: • ↓ triglycerides, total cholesterol, uric acid, urea, SGPT, SGOT • ↑ HDL	[100]
Quetiapine Fumarate | NLC	High shear homogenization	CCRD (13 runs) CMAs: total lipid concentration, surfactant concentration CQAs: PS, PDI, ZP	• Relationship between variables: ↓ PS: ↑ lipid concentration ↑ PS: ↑ surfactant concentration ↓ PDI: ↑ lipid concentration ↑ PDI: ↑ surfactant concentration ↓ ZP: ↑ lipid concentration ↑ ZP: ↑ surfactant concentration • Optimized formulation: 1.2% total lipid, 0.317% surfactant, resulting PS 179.2 nm, PDI 0.22, and ZP −33.63 mV	In vitro drug release study: • Rapid release in FaSSGF pH 1.6 for 3h, followed by slower release in FaSSIF pH 6.5 for the next 4 h • Korsmeyer-Peppas release kinetics model • Non-Fickian drug release	[131]
Raloxifene | NLC	High-shear homogenization	Screening: Taguchi design (7 factors, 8 runs) BBD (17 runs) CMAs: solid lipid concentration, liquid lipid concentration, surfactant concentration CQAs: PS, ZP, EE, DR	• Screening result: solid lipid concentration, liquid lipid concentration, and surfactant concentration were the most influential factors. • Relationship between variables: ↓ PS: ↑ solid lipid concentration, ↑ surfactant concentration↑ PS: ↑ liquid lipid concentration ↓ ZP: ↑ solid lipid concentration, ↑ liquid lipid concentration, ↑ surfactant concentration ↑ EE: ↑ solid lipid concentration, ↑ liquid lipid concentration, ↑ surfactant concentration ↓ DR: ↑ liquid lipid concentration ↑ DR: ↑ solid lipid concentration, ↑ surfactant concentration • Optimized formulation: 794.41 mg solid lipid, 215.4 mg liquid lipid, 1.98% surfactant, resulting PS 186 nm, ZP −23.6 mV, EE 80.09%, and DR 83.87%	In vitro drug release study: • ↓ cumulative drug release (in 0.1% Tween® 80) • Characteristically more sustained than raloxifene tablet • Korsmeyer-Peppas release kinetics model • Fickian drug release In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, AUC, $t_{1/2}$, K_a	[129]
Ritonavir | NLC	Melt emulsification and ultrasonication	CCRD (15 runs) CMAs: total lipid concentration, surfactant concentration CPPs: ultrasound amplitude CQAs: PS, PDI, EE	• Relationship between variables: ↓ PS: ↑ ultrasound amplitude, surfactant concentration ↑ PS: ↑ lipid concentration ↓ PDI: ↑ surfactant concentration ↑ PDI: ↑ ultrasound amplitude, ↑ lipid concentration ↓ EE: ↑ ultrasound amplitude ↑ EE: ↑ lipid concentration, ↑ surfactant concentration • Optimized formulation: 1.82% total lipid, 1.43% surfactant, 40% ultrasound amplitude, resulting PS 187.23 nm, PDI 0.119, and EE 92.01%	In vitro drug release study: • ↑ cumulative drug release (in SGF pH 1.2 for 2h, continued in SIF pH 6.8 for 22 h) • Higuchi release kinetics model • Fickian drug release Ex vivo everted gut sac study: • ↑ apparent permeability coefficient In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, AUC, $t_{1/2}$, relative bioavailability	[13]

Rosuvastatin calcium | SLN	Melt emulsification and solvent diffusion	Screening: Taguchi design (7 factors, 8 runs) I-optimal design (17 runs) CMAs: solid lipid concentration, surfactant concentration CPPs: stirring speed CQAs: PS, ZP, EE, T80%	• Screening result: solid lipid concentration, surfactant concentration, and stirring speed were the most influential factors. • Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ stirring speed ↑ PS: ↑ lipid concentration ↓ ZP: ↑ lipid concentration, ↑ surfactant concentration↑ ZP: ↑ stirring speed ↑ EE: ↑ lipid concentration, ↑ surfactant concentration, ↑ stirring speed ↓ T80%: ↑ lipid concentration, ↑ surfactant concentration, ↑ stirring speed • Optimized formulation: 412 mg solid lipid, 6.25% surfactant, 2625 rpm stirring speed, resulting PS 63.5 nm, ZP −25.5 mV, EE 89.5%, and T80% 8.01 h	In vitro drug release study: • In all fomulations, more than 80% drugs were released within 12 h • Formulation with smaller amount of lipid and larger amount of surfactant showed faster release, while medium amount of lipid resulted in a relatively slower release • Different release kinetics were recorded (Fickian to non-Fickian In vitro gastrointestinal stability • ↔ PS, ZP, EE In vitro cell uptake study: • ↑ Caco-2 cell uptake In vitro cellular permeability study: • ↑ apparent permeability coefficient In situ intestinal perfusion study: • ↑ drug permeability and drug absorption parameters In vivo pharmacokinetic study: • ↑ C_{max}, $t_{1/2}$, AUC, MRT • ↓ T_{max} In vivo pharmacodynamic study: • ↓ triglycerides, total cholesterol, LDL	[101]
Silybin | SLN	Emulsification and solvent evaporation	BBD (15 runs) CMAs: solid lipid concentration, surfactant concentration CPPs: homogenization time CQAs: PS, ZP, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration (at lower amount); solid lipid and homogenization time were not significantly influence PS ↑ ZP: ↑ surfactant concentration; solid lipid and homogenization time were not significantly influence ZP ↓ EE: ↑ surfactant [concentration] ↑ EE: ↑ lipid concentration; homogenization time was not significantly influence EE • Optimized formulation: 400 mg solid lipid, 0.56% surfactant, 10 min homogenization, resulting PS 271.84 nm, ZP −23.78 mV, and EE 70.49%	In vitro drug release study: • Released drug was fewer in HCL pH 1.2, and increased up to 40% in PBS pH 6.8 • Higuchi release kinetics model • Non-Fickian drug release In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, $t_{1/2}$, AUC, MRT, relative bioavailability	[55]
Sucupira Oil | NLC	High pressure homogenization	FFD (7 runs)CMAs: solid lipid concentration, surfactant concentrationCQAs: PS, PDI, ZP	• Relationship between variables: ↑ PS: ↑ lipid concentration, ↑ surfactant concentration↓ PDI: ↑ lipid concentration ↑ PDI: ↑ surfactant concentration ↓ ZP: ↑ surfactant concentration ↑ lipid concentration • Optimized formulation: 0.5% sucupira oil, 4.5% solid lipid, 1.425% surfactant, resulting PS 148.1–159.3 nm, PDI 0.274–0.305, and ZP close to zero	In vitro drug release study: • • Sustained release in PBS pH 7.4 • First-order release kinetics model In vitro cytotoxicity study: • ↔ Caco-2 cell viability	[122]

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Table 2 (Continued).

Drug (s) | LNP Type	Preparation Method	DoE	DoE Results	Performances of Optimized LNP	Ref.
Sulforaphane | NLC	Melt emulsification and ultrasonication	BBD (17 runs) CMAs: total lipid concentration, surfactant concentration CPPs: sonication time CQAs: PS, EE, DL	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ sonication time ↑ PS: ↑ lipid concentration ↓ EE: ↑ surfactant concentration, ↑ sonication time ↑ EE: ↑ lipid concentration ↓ DL: ↑ surfactant concentration, ↑ sonication time ↑ DL: ↑ lipid concentration • Optimized formulation: 3% total lipid, 2.5% surfactant, 4 min sonication, resulting PS 145.38 nm, EE 84.94%, and DL 14.82%	In vitro drug release study: • ↑ cumulative drug release (in SIF pH 6.8) • Higuchi release kinetics model Ex vivo gut permeation study: • ↑ apparent permeability coefficient In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, AUC, $t_{1/2}$, relative bioavailability • ↓ K_{el}	[125]
Telmisartan | NLC	Melt emulsification and ultrasonication	BBD (17 runs) CMAs: total lipid concentration, surfactant concentration CPPs: sonication time CQAs: PS, PDI, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ sonication time ↑ PS: ↑ lipid concentration ↓ PDI: ↑ surfactant concentration, ↑ sonication time ↑ PDI: ↑ lipid concentration ↓ EE: ↑ surfactant concentration ↑ EE: ↑ lipid concentration, ↑ sonication time • Optimized formulation: 2% total lipid, 4% surfactant, 20 min sonication, resulting PS 172.5 nm, PDI 0.272, and EE 83.72%	In vitro drug release study: • • ↑ cumulative drug release (in SGF pH 1.2) • ↑ cumulative drug release (in SIF pH 6.8) • Korsmeyer-Peppas release kinetics model • Fickian drug release Ex vivo tissue uptake study: • ↑ permeation In vivo pharmacokinetic study: • ↑ C_{max}, T_{max}, AUC, relative bioavailability	[52]
Thymoquinone | SLN	Melt emulsification and ultrasonication	BBD (15 runs) CMAs: solid lipid concentration, surfactant concentration CPPs: sonication time CQAs: PS, PDI, EE	• Relationship between variables: ↓ PS: ↑ surfactant concentration, ↑ sonication time ↑ PS: ↑ lipid concentration ↓ PDI: ↑ surfactant concentration ↑ PDI: ↑ lipid concentration, ↑ sonication time ↓ EE: ↑ sonication time ↑ EE: ↑ lipid concentration, ↑ surfactant concentration • Optimized formulation: 3% w/v solid lipid, 2% w/v surfactant, 5 min sonication, resulting PS 166.56 nm, PDI 0.211, and EE 82.66%	In vitro drug release study: • ↑ cumulative drug release rate (in PBS pH 6.8) • Korsmeyer-Peppas release kinetics model • Fickian drug release Ex vivo gut permeation study: • ↑ cumulative permeated drug, apparent permeability coefficient In vitro lipolysis study: • ↑ drug release in aqueous phase and lipid phase GIT retention study: • ↑ drug retained in GIT membrane In vivo pharmacokinetic study: • ↑ C_{max}, $t_{1/2}$, AUC, MRT, relative bioavailability • ↓ T_{max}, K_{el}	[68]
Tilmicosin | NLC	High shear homogenization	Orthogonal design (9 runs) CMAs: stearic acid/oleic acid ratio, surfactant/mixed lipid ratio, drug/mixed lipid ratio, cold/hot water ratio CQAs: PS, EE, DL	• Orthogonal design was applied to examine the importance relativity of factors. • Results indicated that drug/mixed lipid ratio significantly influence PS and DL. • Optimized formulation: 1:9 stearic acid/oleic acid, 30% surfactant/mixed lipid, 30% drug/mixed lipid, 1:1 cold/hot water, resulting PS 276.85 nm, EE 92.92%, and DL 9.14%	In vitro drug release study: • ↓ cumulative drug release (in SGF pH 1.2) • ↓ cumulative drug release (in SIF pH 6.8) In vitro cellular permeability study: • ↓ net efflux ratio • ↑ apparent permeability coefficient in apical-basolateral transport	[62]

Zaleplon | SLN	High shear homogenization and ultrasonication	BBD (17 runs) CMAs: solid lipid concentration, surfactant concentration, cosurfactant concentration CQAs: PS, EE, ZP	• Relationship between variables: ↑ PS: ↑ lipid concentration, ↑ surfactant concentration, ↑ cosurfactant concentration ↓ EE: ↑ surfactant concentration ↑ EE: ↑ lipid concentration ↑ ZP: ↑ lipid concentration, ↑ surfactant concentration • Optimized formulation: 132.89 mg solid lipid, 106.7 mg surfactant, 0.2% w/v cosurfactant, resulting PS 219.9 nm, EE 86.83%, and ZP −25.66 mV	In vitro drug release study: • ↓ cumulative drug release (in 0.1 N HCl pH 1.2 for 2h, continued in PBS pH 6.8 for 22 h) In vivo pharmacokinetic study: • ↑ C_{max}, $t_{1/2}$, AUC, MRT • ↔ T_{max}	[107]

Notes: ↑, increased value; ↓, decreased value; ↔ unchanged value.

Abbreviations: AUC, area under the curve; ALP, alkaline phosphatase; BBD, Box-Behnken design; CCD, central composite design; CCRD, central composite rotational design; CMAs, critical material attributes; CPPs, critical process parameters; CQAs, critical quality attributes; DL, drug loading; DOX, doxorubicin; DR, drug release; DRVE, darunavir ethanolate; EE, entrapment efficiency; ETR, etravirine; EXE, exemestane; FA, folic acid; FaSSGF, fasted-state simulated gastric fluid; FaSSIF, fasted-state simulated intestinal fluid; FFD, full factorial design; GEL, gelucire; HDL, high-density lipoprotein; HPH, high-pressure homogenization; HPMC, hydroxypropyl methylcellulose; HSH, high-shear homogenization; IL-1β, interleukin 1β; IL-6, interleukin 6; LDL, low-density lipoprotein; MRT, mean residence time; NLC, nanostructured lipid carrier; PAL, cetyl palmitate; PBS, phosphate-buffered saline; PEG, polyethylene glycol; PDI, polydispersity index; PS, particle size; SGF, simulated gastric fluid; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; SIF, simulated intestinal fluid; SLN, solid lipid nanoparticle; SLS, sodium lauryl sulfate; THY, thymoquinone; TNF-α, tumor necrosis factor α; ZP, zeta potential.

markedly reduced the particle size.[118] This reduction can be attributed to the low viscosity of the liquid lipid, which facilitates the rapid movement of surfactant molecules, effectively preventing aggregation, and promoting the formation of smaller droplets.[164]

Surfactants are another key factor that influences the particle size. A higher surfactant concentration primarily leads to particle size reduction by enhancing the emulsifying capacity, which prevents droplet agglomeration and concurrently stabilizes the dispersion system of the LNPs.[91,106,116] This trend has been consistently reported in numerous studies on SLN and NLC optimization. For example, while maintaining the other variables constant, increasing the surfactant amount from 1% to 3% effectively decreased the particle size from 49 nm to 20 nm, as reported by El-say et al[123] However, contrasting results have been reported in several studies. This can be attributed to the accumulation of surfactant molecules on the nanoparticles, which enveloped the surface excessively, thereby increasing their size.[119,131]

The preparation method also has a direct impact on the LNP particle size, with CPPs generally selected from adjustable parameters specific to each method. Typically, the application of higher energy, represented by the HPH pressure, homogenization speed, or ultrasound amplitude, leads to a reduction in particle size. A similar effect was observed when pre-LNP primary emulsion was subjected to a high-energy process for an extended period during production. For instance, the HPH pressure accelerates the input materials at high energy, where the resulting cavitation force and shear stress effectively break down particles into smaller sizes.[111] Additionally, an increased number of HPH cycles can further reduce the particle size owing to the prolonged exposure to high pressures. As an example, during the preparation of ezetimibe-loaded NLC, Agrawal et al illustrated that increasing the number of HPH cycles from 5 to 7 at 700 bars significantly reduced the particle size from 614.4 nm to 262.0 nm.[120] In the HME method, increased barrel temperature also reportedly decreases SLN particle size, possibly due to the complete melting of lipids and drugs, which effectively lowers the viscosity of the drug-lipid system.[56]

Influences of Independent Variables on Polydispersity Index

The polydispersity index (PDI) is another commonly selected CQA, which is usually measured along with particle size. This describes the particle size distribution, which indicates the dispersion characteristics of LNPs. A monodisperse system with lower PDI is preferable to ensure homogeneous drug delivery and release. Generally, CMAs influence PDI in a manner similar to that of the particle size. Increased solid lipid content may increase LNP viscosity, obstruct uniform particle breakdown, and ultimately lead to a higher PDI.[103] Formulating NLCs with higher liquid lipid and surfactant concentrations can reduce overall viscosity and surface tension, respectively, subsequently reversing this effect, as demonstrated in several studies.[68,109,118,130]

The influence of CPPs on PDI is not as straightforward, as contrasting interactions have been found in multiple reports. On several occasions, prolonged high-energy applications can be beneficial because extended exposure to cavitation energy promotes a low-PDI system with uniformly distributed smaller particles.[103,124,126] However, beyond the optimal point, excessive high-energy exposure may generate heat, disrupting the surfactant layer and diminishing its stabilizing capacity, ultimately resulting in a more polydisperse LNP system.[165] This phenomenon has been observed in several studies that have utilized ultrasonication or the HPH method.[64,104,113,127]

Influences of Independent Variables on Entrapment Efficiency and Drug Loading

In LNP formulations, entrapment efficiency (EE) and drug loading (DL) determine the extent of drug incorporation within the lipid matrix, ensuring effective delivery to target sites. For the most part, a higher amount of lipids results in a higher EE, owing to the greater space available inside the matrix. This effect was particularly pronounced when drug compatibility with a specific lipid was considered. For example, El Assasy et al demonstrated a considerable increase in EE in amisulpride-loaded NLC when the solid lipid was changed from tripalmitin to Gelucire® 43/1. This may be due to the greater compatibility of amisulpride with Gelucire® 43/1 than with tripalmitin. Furthermore, in the same study, it was also observed that increasing the lipid-to-drug ratio from 7:1 to 13:1 resulted in an improvement in EE from 49.50% to 69.06%.[133] A higher lipid concentration may also increase DL, which can be attributed to the reduction in drug expulsion from the lipid matrix. However, it is notable that the DL of LNP with high lipid content may still be low when the drug makes up only a small fraction of the total mass, as reported in several studies.[104,105,130]

Surfactants have also been found to significantly affect EE in several ways. Gilani et al illustrated that surfactant concentration positively influenced EE, as a higher surfactant amount may prevent drug leakage by facilitating layer formation in the aqueous phase.[114] In contrast, in a study on sulforaphane-loaded NLC, Soni et al argued that excessively high surfactant levels could promote drug partitioning into the external aqueous phase by increasing drug solubility, ultimately leading to greater drug expulsion and resulting in low EE. A similar interaction was also observed for DL in the same study.[125]

The influence of CPPs on EE generally followed a converging trend, where a higher applied energy tended to lower EE. For example, in ultrasonication-based preparation, a higher ultrasound amplitude or prolonged sonication time may expose LNPs to greater acoustic cavitation energy, potentially disrupting lipid matrix integrity and reducing their ability to retain the drug, thereby decreasing EE.[13,68] However, contradictory interactions, where an increased EE was achieved with higher energy levels, have also been observed on several occasions. Agrawal et al revealed that high pressure can alter the lipid matrix, creating more space within its imperfect structure and thus enhancing drug incorporation.[120] It has also been reported that during the production of fenofibrate-loaded SLN using the HME method, increasing the screw speed can enhance EE by generating a higher shear force inside the barrel, which promotes the formation of a homogeneous emulsion comprising the drug, lipid, and surfactant.[56]

Influences of Independent Variables on Zeta Potential

From a structural standpoint, a zeta potential (ZP) value above +30 mV or below −30 mV signifies stronger electrostatic repulsion between particles, which helps prevent aggregation and maintains the stability of the LNP dispersion system. Additionally, in oral drug delivery, the surface charge of the LNP may facilitate charge-dependent interactions across GI barriers.[37,166] The excipients used in the LNP influence the zeta potential in various ways. In the optimization of quetiapine fumarate-loaded NLC, Ayed et al used a combination of poloxamer 188 and soybean lecithin as surfactants, resulting in a zeta potential ranging from −36.2 mV to −32.5 mV. The observed anionic surface charge was likely attributed to the phospholipid component of lecithin. Additionally, the study indicated that increasing the surfactant concentration led to a more negative ZP value.[131] Dudhipala et al demonstrated a similar interaction in the preparation of zaleplon-loaded SLN using poloxamer 188 and egg lecithin. Their polynomial equation analysis further revealed that poloxamer 188 had an insignificant impact on the ZP.[107]

In functionalized LNPs, the use of charge-modifying excipients evidently influences ZP. During optimization of carvedilol-loaded SLN, El say et al demonstrated that increasing the concentration of stearylamine, a positive-charge modifier, from 4% to 6% markedly elevating the ZP from +8.4 mV to +25.2 mV.[123] El-Dakroury et al used chitosan with various molecular weights as a coating agent for fexofenadine-loaded SLN with the objective of improving the mucoadhesion property of the nanoparticles. These findings indicate that changing the chitosan type from low to high molecular weight increases the positive value of ZP to some extent, which may be due to the higher density of the positively charged amino groups in chitosan.[48,167]

Influences of Independent Variables on Drug Release

For oral administration of LNPs, drug release studies are typically conducted either directly as a CQA during optimization or evaluated separately after the optimized formulation has been established. It was found that a specific variable may exhibit different interactions with drug release. Several reports have indicated that a higher solid lipid concentration results in a lower amount of released drug, which can be attributed to the increased viscosity and lipid core thickness, thus hindering drug expulsion from the matrix.[115,123] Contrasting results have been observed in other studies, in which the addition of solid lipids increased the cumulative drug release percentage. This effect may be attributed to prolonged drug retention within the matrix, allowing for gradual release, which ultimately results in a higher cumulative release at a certain time period.[109,129]

Surfactants also variably influence the LNP drug release. A higher surfactant concentration can improve drug release by enhancing emulsification, reducing particle size, and increasing surface area, which collectively facilitates drug release. However, an excessive amount of surfactant may accumulate on the particle surface, leading to an increase in size and ultimately slowing the drug release, as reported in several studies.[108,130]

A direct correlation between CPPs and drug release has been explored less frequently, as most studies that consider drug release as a CQA have primarily focused on optimizing CMAs. In the optimization of dapagliflozin-loaded NLC, Zafar demonstrated that an increased number of HPH cycles positively influenced drug release, likely due to reduced particle size, which ultimately increased the surface contact area with the release medium.[115] Similarly, in a study on SLN containing rosuvastatin calcium, it was observed that a shorter time was required for 80% drug release when stirring speed was increased, which may also be attributed to reduced particle size.[101]

It is also noteworthy that unlike other dependent variables such as particle size, PDI, EE, and ZP, where the desirable values are well-defined (particle size and PDI should be low, ZP should exceed ±30 mV, and EE should be high), the preferred drug release profile can vary among investigators. For instance, in the development of NLC containing olmesartan medoxomil, Beg et al demonstrated the positive impact of surfactant concentration on the quantity of drug released within 4 h (Q_{4h}), where the minimum value was considered optimal because of the goal of achieving sustained release.[132] A similar surfactant-drug release interaction was also observed in a study of abiraterone acetate-loaded SLN by Konatham et al. However, they were optimized for a maximum value owing to their selection of a 24-hour release period (Q_{24h}), as it is more favorable for obtaining a higher cumulative release.[109] Hence, when considering drug release as a CQA, the optimization direction can vary depending on the assigned constraints, despite the same factor-response interaction.

Performance of Optimized Lipid Nanoparticles for Oral Drug Delivery

The ultimate objective of QbD-driven LNP formulation is to develop a high-performance product tailored to the intended use of the drug. Thus, it is essential to investigate the performance of LNPs optimized through DoE to ensure that they satisfy a predefined target profile. This performance is intrinsically governed by CQAs, such as particle size, polydispersity index, zeta potential, encapsulation efficiency, drug loading, and release profile, each of which is influenced by specific CMAs and CPPs identified during the QbD process. The performance of optimized LNPs for oral drug delivery is typically evaluated using in vitro, in vivo, or ex vivo methods across various aspects, such as drug release kinetics, permeability, pharmacokinetics, and therapeutic efficacy (Table 2).

Drug Release and Kinetics

Optimized LNPs should exhibit a controlled and sustained drug release to ensure prolonged therapeutic action. In vitro drug release studies, often conducted using dialysis membrane techniques, help to assess the release profile under simulated physiological conditions. Low- and high-pH settings are commonly implemented in specific media to evaluate release profiles in both gastric and intestinal environments. For example, an acyclovir-loaded SLN drug release study was conducted in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8) for 24 h. The results indicated that the release rates in both media were lower than that of free acyclovir, suggesting a sustained release pattern for 24 h. It is also displayed that the release plots in both media were superimposed, implying that acyclovir release from the optimized SLN was not pH-dependent.[106]

It is also common to evaluate drug release under different pH conditions as a continuation, rather than as a separate measurement, where the release profile is depicted as a single continuous plot. This is typically conducted by first assessing the drug release in a low-pH medium for 2 h, followed by a transition to a high-pH medium for the remaining 10 or 22 h, assuming that the gastric transit time is shorter than the intestinal transit time.[107] A biphasic release pattern is frequently observed, characterized by a rapid initial release in an acidic medium, followed by sustained release under higher pH conditions. The initial burst release occurred due to the presence of the adsorbed drug on the LNP surface, which was readily released upon contact with the medium, whereas the subsequent sustained release was governed by the gradual diffusion of the encapsulated drug from the lipid matrix.[13,112,121,133] For instance, in a drug release study of pioglitazone from SLNs, Shaveta et al demonstrated that more than 30% of the drug was released within the first 2 h, whereas a sustained release of up to 89.56% was achieved after 24 h. Furthermore, the study revealed that the cumulative release of pioglitazone from the optimized SLN was significantly higher than that of the pure drug under the same conditions, which reached only 43.12%.[100]

Several mathematical models, including zero-order, first-order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas models, are commonly used to analyze drug release kinetics. The coefficient of determination (R^2) for each model was subsequently

examined to determine the best fit, allowing for a more precise interpretation of drug release behavior. Vieira et al demonstrated that the release kinetics of sucupira oil from the NLC matrix were best described by the first-order model ($R^2 = 0.9829$), compared to the zero-order ($R^2 = 0.7722$), Higuchi ($R^2 = 0.9268$), and Korsmeyer-Peppas ($R^2 = 0.8403$) models. This indicates that the release process is primarily governed by the concentration gradient of sucupira oil over time.[122] Conversely, zero-order release kinetics were observed in an Eudragit-coated SLN containing linagliptin, demonstrating that drug release occurred at a constant rate, independent of the remaining drug concentration. This may be attributed to the pH-responsive Eudragit coating, which prevents premature drug release in the stomach and dissolves in the intestine, allowing for controlled water penetration and diffusion. The lipid matrix further regulates the drug release, prevents burst effects, and ensures a controlled drug profile.[46] In another study, release kinetics were best described by the Higuchi model, which is primarily governed by diffusion through a lipid matrix.[55,111,116,125] The Hixson-Crowell model best fits highly uniform LNPs, where drug release is driven by dissolution following a gradual reduction in particle size, as demonstrated in a study of carvedilol-loaded SLN.[123]

Several studies have also reported that the Korsmeyer-Peppas model is the most suitable for describing release kinetics, which is predominantly regulated by a combination of multiple mechanisms such as swelling, diffusion, and lipid matrix erosion.[52,68,124] Additionally, the exponent value (n) calculated from the Korsmeyer-Peppas equation provides further insight into the drug release mechanisms. These are generally classified as Fickian diffusion ($n < 0.45$), non-Fickian diffusion or anomalous transport ($0.45 < n < 0.85$), and case II transport ($n = 0.85$).[115,130] For example, the n values for quetiapine fumarate (QTF) released from NLC in fasted-state simulated gastric fluid (FaSSGF) and fasted-state simulated intestinal fluid (FaSSIF) were 0.508 and 0.624, respectively. The lower n value in FaSSGF, which is closer to Fickian diffusion, suggests that QTF release in the gastric environment may be primarily governed by simple diffusion from the outer shell of the NLC, with a partial contribution from erosion. Conversely, the higher n value in FaSSIF indicates that intestinal drug release is likely driven by a combination of non-Fickian diffusion and erosion mechanisms.[131] A study by Beg et al illustrated similar findings to some extent, demonstrating that the release mechanisms of rosuvastatin calcium from SLN range from Fickian to non-Fickian diffusion. The matrix-type structure of SLN plays a crucial role in the regulation of drug diffusion. Furthermore, the release kinetics exhibited a direct relationship with solid lipid concentration, which negatively affected the diffusional drug release mechanism. A higher lipid content favored the predominance of non-Fickian diffusion, suggesting that an increase in lipid levels contributed to a greater influence of erosion-based release mechanisms.[101]

Drug Permeability and Intestinal Absorption

Effective oral drug delivery requires sufficient permeability of intestinal epithelium. Optimized LNP formulations may enhance drug permeability by modulating P-gp efflux or by utilizing lipid-mediated transport mechanisms. Several methods, such as ex vivo gut permeation models and in vitro cell line studies, are frequently used to evaluate drug permeability and transport across the intestines.

In gut permeation studies, rat or goat intestines are frequently used as models, employing either everted or non-everted techniques. The everted method involves turning the intestine inside out. The mucosal side faces outward, whereas the serosal side faces inside, allowing direct access to the absorptive surface. Meanwhile, the non-everted method maintains the intestine in its natural orientation, where the mucosal surface remains inside.[168] In an everted gut sac study of ritonavir-loaded NLC, it was revealed that the apparent permeability coefficient (P_{app}) of the optimized formulation was markedly higher than that of the pure drug suspension.[13] In another study, a non-everted gut sac method was used to compare the permeability of an optimized NLC-containing efavirenz with that of the free drug. The sample was placed on the mucosal side and aliquots were withdrawn from the serosal compartment at predetermined time intervals. The results showed a two-fold increase in both the cumulative permeated drug and P_{app} of the NLC, confirming the potential of lipid-based nanocarriers to enhance intestinal permeability.[130]

In vitro cellular permeability studies offer a deeper understanding of the drug transport across the intestinal barrier. Caco-2 cells are commonly used as models in these studies because of their morphological and functional similarities to the human intestinal epithelium, making them reliable for assessing drug permeability and absorption.[122] In a permeability study using the Caco-2 cell line, an optimized SLN formulation reportedly increased the P_{app} of lurasidone HCl six-fold compared to that of the pure drug. Further analysis was performed to elucidate the transport mechanism, using

nystatin and chlorpromazine as inhibitors of caveolae-mediated endocytosis and clathrin-mediated endocytosis, respectively. These findings suggest that both transport pathways play a significant role in drug uptake across Caco-2 cells.[112] In a transport study of rosuvastatin calcium-loaded SLN, filipin and sucrose were utilized as inhibitors of caveolae-mediated and clathrin-mediated endocytosis, respectively. The reduction in drug uptake was significant only in the presence of sucrose, suggesting that the transport of rosuvastatin calcium across Caco-2 cells is likely clathrin-mediated.[101]

Besides Caco-2 cells, other cell lines, such as Madin-Darby canine kidney (MDCK) cells, can also serve as epithelial models. MDCK cells are particularly useful for studying paracellular transport owing to their distinct tight junction properties. In a cellular permeability study of tilmicosin-loaded NLC using the MDCK cell model, the optimized formulation exhibited a bidirectional transport mechanism, enhancing cellular uptake while simultaneously inhibiting efflux transport. Additionally, this study confirmed that drug uptake is primarily mediated through caveolae-dependent endocytosis, highlighting the role of LNPs in facilitating transcellular absorption.[62]

Pharmacokinetic Profile and Bioavailability

One of the primary advantages of LNPs is their ability to improve the pharmacokinetic profiles of poorly soluble drugs. Parameters such as the maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}), area under the curve (AUC), and relative bioavailability are critical indicators of systemic drug exposure. Comparative in vivo pharmacokinetic studies between the optimized LNP formulation and pure drug suspensions or marketed drugs will help to validate the effectiveness of the experimental design. For instance, the optimized formula of NLC-based capsules containing amisulpride demonstrated a significantly higher C_{max} and AUC than the reference drug Amipride®. This extended to a 2.52-fold increase in relative bioavailability, highlighting the potential of NLC formulations to enhance the oral absorption and overall systemic availability of amisulpride.[133] It is also notable that other pharmacokinetic parameters, such as the elimination rate constant (K_{el}), can provide further insight into the fate of the drug prior to entering the systemic circulation. Several studies have reported a decrease in K_{el} for encapsulated drugs compared to free drugs, suggesting prolonged exposure, slower elimination, and enhanced absorption. This also demonstrates the ability of LNPs to facilitate lymphatic uptake, allowing the drug to bypass the first-pass metabolism.[102,116,169]

The role of LNP formulations in facilitating lymphatic transport has often been investigated using the chylomicron flow block model. In this approach, cycloheximide is administered to inhibit chylomicron formation, allowing researchers to differentiate between the lymphatic and non-lymphatic drug absorption pathways. The effect of lymphatic transport on systemic drug exposure was assessed using the pharmacokinetic profile of each formulation. Rangaraj et al investigated the lymphatic uptake capability of ibrutinib-loaded NLC. The resulting C_{max} and AUC of the NLC without cycloheximide were each 2.75-fold and 3.57-fold higher compared to NLC that administered along with cycloheximide.[119] Cycloheximide inhibits chylomicron secretion, blocking systemic entry through the lymphatic pathway and ultimately leading to lower plasma concentrations. Several reports of drug-loaded LNPs have consistently shown similar results, confirming their potential to enhance lymphatic transport.[64,124,170]

Therapeutic Efficacy

Following the QbD-based preparation, optimized LNPs should demonstrate improved therapeutic outcomes. Based on the predefined QTPP and inherent characteristics of the loaded drug, further in vitro or in vivo studies are commonly conducted to assess the efficacy of the formulation in achieving the desired pharmacological effects.

Inflammatory Disorders Treatment

Based on in vivo studies in rats, orally administered bergenin and nabumetone encapsulated in NLC displayed a significant reduction in carrageenan-induced paw edema compared to the pure drug suspension, revealing the superiority of lipid-based nanoformulations as anti-inflammatory agents. Furthermore, the optimized bergenin-loaded NLC exhibited a more prolonged effect than the standard drug indomethacin, which was attributed to higher absorption and longer circulation time.[102,113]

The anti-inflammatory potential of drug-loaded LNPs may further elucidate their efficacy for specific disorders. In rheumatoid arthritis, pro-inflammatory cytokines such as TNF-α and IL-1β promote the formation of osteoclasts, which

are specific cells responsible for bone destruction.[171] The downregulation of these cytokines is a key target in arthritis therapy as it helps mitigate inflammation and preserve joint integrity. An in vivo study on apigenin-loaded SLN demonstrated that the optimized formulation significantly reduced TNF-α and IL-1β levels compared to pure apigenin. This orally administered formulation enhances systemic absorption and distribution to tissues and bones, leading to higher drug concentrations in the synovial joint, highlighting the potential of SLN as an anti-arthritic agent.[114] A similar pattern was observed in a study on SLN containing fexofenadine HCl for ulcerative colitis therapy. Oral administration of this formulation evidently decreased TNF-α and IL-6 levels in rat colon tissue by 70.79% and 72.99%, respectively. Moreover, optimized SLN also downregulated phosphatidylinositol-3 kinase (PI3K) and protein kinase B (Akt), both of which play crucial roles in the inflammatory signaling pathways implicated in the pathogenesis of ulcerative colitis.[48]

Lipid-Lowering Treatment

Optimized LNPs have also been shown to enhance the therapeutic potential against hyperlipidemia. In separate studies, ezetimibe-loaded NLC and rosuvastatin calcium-loaded SLN reportedly reduced total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) levels and increased high-density lipoprotein (HDL) levels in rats treated with a high-fat diet (HFD).[101,120] Similar improvements in the lipid profile have been observed in studies investigating the antidiabetic potential of dapagliflozin-loaded and pioglitazone-loaded SLNs. In these studies, streptozotocin was administered along with HFD to induce diabetes. It was revealed that, in addition to lowering TC, TG, and LDL levels while increasing HDL, the optimized SLN formulations also reduced SGOT, SGPT, ALP, and blood glucose levels, confirming the potential benefits of LNPs in liver function regulation.[100,108]

Cancer Treatment

The use of LNPs for oral administration in cancer treatment has attracted considerable interest because of their prolonged circulation and ability to achieve site-specific targeting. Moraes et al developed an NLC for the oral delivery of doxorubicin and assessed its potential in breast cancer treatment. The optimized DOX-NLCs, functionalized with PEG and folic acid, demonstrated enhanced cytotoxicity against MDA-MB-231 cells and exhibited rapid cellular internalization with over 80% uptake within 30 min, highlighting its superior cellular uptake efficiency.[49] Similar performance was observed in lycopene-loaded NLC, where cell death was higher than that of the free drug. This may be attributed to higher lycopene endocytosis and subsequent accumulation within cells.[126] Another breast cancer model, MCF-7 cells, was employed to elucidate the potential of NLC to deliver exemestane and thymoquinone (EXE-THY). The EXE-THY-loaded NLC simultaneously demonstrated higher cellular uptake and higher cytotoxicity against MCF-7 cells, as illustrated by the significantly lower IC_{50} compared to each drug and their combination. The sustained release of EXE-THY from the NLC is particularly beneficial for extending the drug exposure toward cancer cells.[103]

The anticancer potential of the optimized LNPs has also been evaluated in a prostate cancer model. Sabale et al formulated NLC containing flutamide, a nonsteroidal anti-androgen commonly used in prostate cancer treatment. However, unlike the conventional approach in which the performance of the LNP formulation is compared with that of the free drug, this study aimed to investigate whether nanonization affects the therapeutic activity of flutamide. The results showed that PC3 cell viability upon treatment with flutamide-loaded NLC was comparable to that of free flutamide, but remarkably lower than that of blank NLC, confirming that nanonization did not compromise the anticancer activity of the drug. The authors suggested that incorporating flutamide into the NLC matrix is especially advantageous for facilitating chylomicron formation, which enhances lymphatic uptake and ultimately enables a more efficient oral delivery for prostate cancer therapy.[127]

HIV Treatment

A major challenge in HIV treatment is the persistence of viral reservoirs, which allow the virus to evade conventional antiretroviral therapy. Poor oral bioavailability further complicates effective drug delivery because many antiretroviral drugs have low solubility, rapid metabolism, and limited penetration into these reservoirs.[172] The use of LNP formulations for antiretroviral drugs has been increasingly explored to address these challenges. In a QbD-based study, Muheem et al developed a dual drug-loaded NLC containing etravirine and darunavir ethanolate functionalized with d-α-tocopheryl polyethylene glycol succinate (TPGS). An in vitro study in TZM-bl cell lines showed that the optimized

NLC formulation inhibited HIV-1 infection by 50% at a drug concentration 58 times lower than that of the pure drug suspensions. Furthermore, the results confirmed chylomicron formation and P-gp efflux inhibition by TPGS in the NLC formulation, highlighting its potential as an oral antiretroviral therapy.[124]

Schizophrenia Treatment

Owing to the chronic nature of psychotic disorders, long-term treatment is often necessary to manage the symptoms and prevent relapse.[173] Developing an effective oral formulation for psychotic therapies is crucial to enhance patient compliance and reduce dosing frequency, thereby minimizing the risk of adverse effects, including drug-induced Parkinsonism, tardive dyskinesia, and metabolic disturbances.[174] Aligning with this objective, Patel et al developed an SLN formulation containing lurasidone HCl to evaluate its efficacy in the treatment of schizophrenia in MK-801-induced rats. The optimized SLN significantly reduced the escape latency during the pole-climbing test, indicating improved cognitive function. Furthermore, the same formulation demonstrated a reduction in extrapyramidal side effects after 7−21 days of catalepsy testing, further highlighting its potential to effectively manage schizophrenia, while minimizing motor-related adverse effects.[112]

Challenges and Future Perspectives

Despite the advantages of QbD in the development of LNPs for oral drug delivery, several challenges persist, particularly the formulation complexity, scalability, and regulatory acceptance. One of the primary difficulties lies in the nonlinear relationship between formulation variables, including CMAs, CPPs, and CQAs, which often makes optimization unpredictable Although DoE helps to systematically analyze these interactions, the experimental workload and associated costs remain significant. To address this, future advancements can focus on the integration of artificial intelligence (AI) into QbD modeling. As part of AI technology, machine learning tools, such as artificial neural networks (ANN), can analyze the complex relationships between the properties of drugs, excipients, and process parameters. This leads to an accurate prediction of the formulation performance in pharmacokinetics/pharmacodynamics (PK/PD) studies, enabling a more efficient approach in the optimization step during pharmaceutical manufacturing.[175,176]

Another key challenge is the scalability of the optimized formulations. Many LNP formulations that perform well at laboratory scale fail to retain their properties during large-scale manufacturing. Conventional preparation techniques, such as HPH, HSH, and ultrasonication, may not always be feasible for industrial-scale production, owing to batch-to-batch variations and process inefficiencies. Continuous manufacturing and Process Analytical Technology (PAT) should be emphasized to ensure real-time monitoring of particle size, zeta potential, and encapsulation efficiency, reducing batch inconsistencies while improving process efficiency.[144,177,178]

From a regulatory perspective, nanoformulations face evolving challenges owing to the lack of standardized evaluation criteria for safety, bioavailability, and quality control. Although ICH guidelines outline the general principles for QbD-driven pharmaceutical development, a globally harmonized framework for lipid-based nanomedicines is still lacking. This regulatory uncertainty can delay clinical translation and commercialization.[179] The establishment of regulatory standards across different regions to streamline approval processes can ensure safety compliance and ascertain bioequivalence assessment protocols for LNP-based drug delivery systems.[180]

The future of QbD-driven LNP development will also be shaped by personalized medical approaches. The current formulations are designed for broad patient populations; however, individual metabolic variations can significantly affect drug absorption, distribution, and therapeutic efficacy. Integrating pharmacogenomics and precision medicine principles into LNP design can lead to tailored drug delivery systems that optimize therapeutic outcomes for individual patients.[181] Furthermore, nanoinformatics and high-throughput screening techniques can be employed to refine LNP formulations based on patient-specific factors such as genetic makeup, microbiome composition, and disease pathology.[182]

Conclusion

The QbD framework has emerged as a powerful approach for the systematic development and optimization of LNPs for oral-drug delivery. Given the challenges of oral administration, including gastric degradation, enzymatic metabolism, limited intestinal permeability, and first-pass metabolism, SLNs and NLCs have demonstrated significant potential for

improving drug bioavailability and therapeutic outcomes. Unlike traditional one-factor-at-a-time (OFAT) methods, QbD enables a structured approach through DoE, allowing CMAs, CPPs, and CQAs to be identified, modeled, and optimized. This facilitates fine control over key properties, such as particle size, drug encapsulation, zeta potential, and release behavior, supporting the development of reproducible and high-performance formulations.

Despite these advancements, challenges remain in the clinical translation, regulatory harmonization, and industrial scalability of oral LNPs. Future directions include the integration of artificial intelligence in QbD-based optimization, the adoption of continuous manufacturing techniques, and alignment of regulatory standards across regions. Moreover, the application of QbD in the context of personalized medicine offers new possibilities for individualized oral therapies. Overall, the integration of QbD principles in LNP optimization not only enhances drug performance but also ensures a more reliable, scalable, and compliant approach to the development of oral drug delivery systems. Continuous innovations in this field have immense potential to improve patient outcomes and expand the therapeutic landscape of nanomedicine.

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