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REVIEW

Optimization of nanostructured lipid carriers: understanding the types, designs, and parameters in the process of formulations

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Abstract Lipid-based nanoparticles for drug delivery have been employed in the development of nanomedicine for various applications. One such versatile nanoparticle type is nanostructured lipid carriers (NLCs) that include multiple combinations of lipids and drugs for diverse routes of administrations. Optimization of NLCs to achieve ideal particle size distribution, dispersion in the aqueous environment, long-term stability, drug protection ability, and targeting features is necessary for designing improved drug formulations. However, very few studies have attempted to discuss explicitly the sequential requirements for optimization. Besides, several compositional variables can confound the design of an NLC drug formulation, making it essential for critical evaluation of factors that affect the NLC's physicochemical properties. Therefore, this review intends to discuss the multi-step process taken during optimization and highlight the components, methods, statistical designs, trends observed between the variables, and modifications of NLCs for targeted delivery, with the objectives of improving efficiency and increasing success rates in drug delivery studies.

Keywords Nanostructured lipid carrier · Solid lipid nanoparticle · Optimization · Design of experiments · Targeted drug delivery

Abbreviations

BBD	Box-Behnken design
CCD	central composite design
DLS	dynamic light scattering
EPR	enhanced permeation reaction
HLB	hydrophile-lipophile balance
LCT	long-chain triglyceride
MCT	medium-chain triglyceride
MPS	mononuclear phagocyte system
NLC	nanostructured lipid carrier
PDI	polydispersity index
PEG	polyethylene glycol
PLGA	poly (lactic-co-glycolic) acid
PVA	polyvinyl alcohol
RSM	response surface methodology
TCA	taurocholic acid
WGA	wheat germ agglutinin

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Introduction

Nanostructured lipid carrier (NLC) is the enhanced version of solid-lipid nanoparticle (SLN), a type of lipid-based drug delivery system. In contrast to SLNs, NLCs are composed of solid and liquid lipid to increase drug loading capacity and prevent initial drug burst release (Müller, Radtke, and Wissing 2002; Muller, Radtke, and Souto 2005; Muller, Shegokar, and Keck 2011) (Fig. 1). In recent years, NLCs have been exploited as drug delivery carriers to targeted regions using diverse routes of administration. For instance, NLCs for topical delivery are widely utilized in cosmetics and dermatological treatments (Pardeike, Hommoss, and Müller 2009; Zsikó et al. 2019). Besides that, oral administration of NLCs for inflammatory bowel disorders is extensively studied due to their prolonged residence time in the gastrointestinal tract (Liu et al. 2014). As for chemotherapy, anti-cancer drugs such as doxorubicin and paclitaxel have been encapsulated in NLCs to be delivered through the pulmonary route (Taratula et al. 2013).

The reason behind the implementation of nano-scaled drug delivery systems is the hydrophobicity of several active compounds intended for targeted delivery in vivo (Gordillo-Galeano and Mora-Huertas 2018). Lack of drug dissolution in the aqueous biological environment results in poor absorption and low oral bioavailability (Khan et al. 2015). It also causes non-specific accumulation in unwanted regions of the body and an abnormal pharmacokinetic profile that may prevent eventual excretion by the kidneys (Trapani et al. 2012). To overcome these problems, NLCs with enhanced dissolution rate are formulated as drug carriers. This results in improved bioavailability of the drug in question and increased specificity in targeted delivery (Müller, MaÈder, and Gohla 2000). Several studies have shown better therapeutic response when a drug is delivered via NLCs as compared to when it is delivered as a standalone (Beloqui et al. 2016). Consequently, numerous distinct NLC formulations are being explored to deliver a variety of active compounds.

Formulation of NLCs is not a difficult task, but optimization is necessary to produce NLCs with the best characteristics. An optimized nanocarrier should come with traits such as apparent solubility in the biological environment, long biological half-life, biocompatibility, drug-protecting capabilities, ability to bind to target regions, and simple intracellular penetration (Pathak and Thassu 2016). These features ensure that the encapsulated drug can be carried by a biocompatible carrier through an aqueous environment without degradation for a prolonged period, before reaching the targeted sites to be endocytosed into the cells. The formulations should effectively exhibit these traits in vivo and therefore measurable characteristics such as particle size, polydispersity index, zeta potential, entrapment efficiency, drug release mechanism, and storage stability are extensively studied in vitro.

Particle size and polydispersity index affect the dissolution of nanoparticles in an aqueous environment while zeta potential highly affects the stability of the system for storage and systemic circulation (Pathak and Thassu 2016). Moreover, entrapment efficiency and drug release mechanisms demonstrate the robustness of the carrier (Abouelmagd et al. 2015; Gordillo-Galeano and Mora-Huertas 2018). Additional features such as targeting and cell-penetrating abilities are dependent on the modifiers used on the nanoparticle surface (Bar-Zeev, Livney, and Assaraf 2017). To simultaneously optimize the different characteristics with minimal efforts and costs, response surface methodology (RSM) has been used. RSM is a collection of mathematical and statistical techniques in which a number of independent variables are examined to determine the responses, specifically the measurable characteristics of the NLC (Khuri and Mukhopadhyay 2010). These characteristics will be discussed in detail in the "Desired characteristics and associated variables" section.

Sequential optimization emphasizes on preliminary materials and methods selections, design of experiments, manipulation of formulation parameters via statistical designs, and modifications of NLCs. Meticulous examination of these steps can result in the formation of an ideal NLC encapsulating individual drugs for effective and efficient delivery. It is important to note that there are various lipids and drug combinations that may give different responses, but generally, these characteristics can be forecasted within a certain range, if we carefully regulate the factors that may affect them. Overall, this article aims to consolidate relevant studies to summarize the optimization steps taken in nanoparticle formulation, which are selections of materials, methods, and statistical designs, followed by evaluation of factors that affect the NLC's physicochemical properties, stability, and targeting ability. By providing systematic guidance, the review attempts to avoid trial and error approach in the design of formulations.

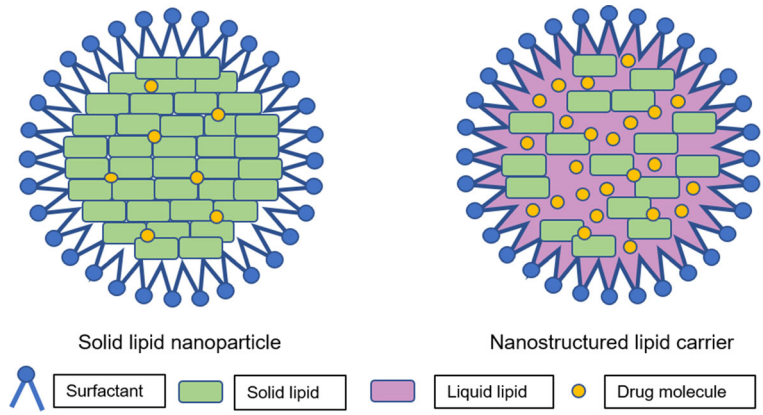


Fig. 1 Schematic illustrations of a rigid solid lipid nanoparticle with low drug load versus a flexible nanostructured lipid carrier with high drug load

Preliminary optimization

Selection of solid and liquid lipids

The lipids used in NLC formulation can exist as a solid or a liquid at room temperature. Several different lipids have been used in NLC formulation and these are selected primarily based on drug-lipid compatibility (Bummer 2004). Compatibility or specifically the solubility of a hydrophobic drug in lipid can be visualized physically or measured spectrophotometrically (Joshi et al. 2008; Liu et al. 2012). In the case of solid lipids with high melting points, the drug is dissolved with the solid lipid until a visible saturation point is observed. Analysis with liquid lipid, however, requires measurement of absorbance at specific wavelengths to determine the amount of drug dissolved in the lipid. The higher the amount of drug dissolved, the more compatible it is with the lipid, which will subsequently be selected as a component of the lipid matrix (Joshi and Patravale 2008).

Several studies have reported better solubility of drugs in medium-chain triglycerides (MCT) compared to long-chain triglycerides (LCT). In the solubility studies of isradipine, Emulcire™ 61 was found to be the best solid lipid solvent due to its emulsifying property. Besides, the presence of the –OH group in Emulcire™ 61 and Capryol™ 90 enhanced the formation of a stable complex with the drug (Alam et al. 2018). Similarly, tacrolimus was found to be more soluble in MCT than LCT (Khan et al. 2016). Highly lipophilic drugs such as progesterone and penclomedine too were found to exhibit greater solubility and oral bioavailability in MCT (Christensen et al. 2004; Kaukonen et al. 2004). Some exceptions to these theories disprove the generalization attempted with lipophilic drugs. Instead, these findings have been justified with discussions on the structural interaction that rises between the lipophilic drug and the lipid candidates (Khan et al. 2016).

In most cases, stearic acid and its derivatives are used as solid lipids because stearic acid is a natural fat available in animals and plants, making it biocompatible and safe to be used for drug delivery (Gonzalez-Mira et al. 2010; Kelidari et al. 2017a). Additionally, stearic acid is recommended to carry anti-cancer drugs due to its ability to penetrate cancer cells and accumulate in the cytoplasm (Severino et al. 2011; Miao et al. 2012). Palmitic, lauric, behenic, oleic, and myristic acid derivatives make up the rest of the fatty acid–based solid lipids. These fatty acids are also naturally found in animals and plants; hence, they are biologically safe to be used in drug delivery. In some cases, hard fats and waxes can become constituents of the lipid matrix (Ferreira et al. 2015; Monteiro et al. 2017). Hard fats are the products of hydrogenating unsaturated oils, while waxes are extracted from animals or plants. Occasionally, combinations of different solid lipids are used to reduce the crystallinity of the lipid matrix to increase drug load. These combinations are made up of fatty acids and fats or waxes (Kudarha et al. 2015; Garcia-Orue et al. 2016).

As for liquid lipids, the most frequently used fatty acid is oleic acid which is naturally found in animals and plants. Its lack of color and odor makes it suitable to be used in drug delivery (Velmurugan and Selvamuthukumar 2015; Dudhipala, Janga, and Gorre 2018). Monoglycerides and diglycerides of fatty acids are also employed, and these are obtained either naturally from plant seeds or synthesized through

glycerolysis of triglycerides. For example, Capmul® MCM is a mixture of monoglycerides and diglycerides of medium-chain fatty acids (Joshi and Patravale 2008; Negi, Jaggi, and Talegaonkar 2013; Rajput et al. 2018). Triglycerides are formed from esterification of glycerol and three molecules of fatty acids. Miglyol® 812 is a type of MCT commonly used in many drug delivery vehicles (Garcia-Orue et al. 2016; Senna et al. 2018). Likewise, several natural oils such as castor, sunflower, pomegranate seed, calendula, and soybean oils are found to be compatible with certain drugs in NLC applications (Oliveira et al. 2016; Jawahar et al. 2018; Kraisit and Sarisuta 2018; Pivetta et al. 2018; Soleimanian et al. 2018). A combination of these oils can also be used as liquid lipid components (Yang, Ju, and Dong 2016). Table 1 shows some of the common lipid-drug combinations used in NLC studies.

Selection of surfactants

After lipids selection, appropriate surfactants are considered in the formulation process. Surfactants are surface-active agents that reduce the interfacial tension between the two phases of oil and water, owing to their hydrophilic head and hydrophobic tail. They also confer stability to the product in the final dispersion and during storage. Surfactants can be categorized into four groups, namely cationic (positively charged), anionic (negatively charged), non-ionic (no charges), and amphoteric (charge depends on pH). The selection of surfactant principally depends on the route of administration of the nanoparticle, the hydrophile-lipophile balance (HLB) value of the surfactant, surface modification of the nanoparticle, and the ability to prevent in vivo degradation of the lipid (Attama, Momoh, and Builders 2012; Kaur et al. 2015).

In cases where a theoretical selection of surfactant alone is inadequate, the solubility of the drug in surfactants can be evaluated. Khan and colleagues used an aqueous titration method to determine the surfactant with the best emulsifying property (Khan et al. 2016). Surfactants were added to the melted lipid phase, and double-distilled water was added dropwise to the lipid-surfactant mixture. A ternary phase diagram was then used to determine the best surfactant combination and their ratio (Khan et al. 2016). Similarly, solubility tests of celecoxib with different surfactants were carried out in the optimization of celecoxib-loaded NLCs (Joshi and Patravale 2008).

Surfactants are usually selected based on their HLB values according to the types of emulsion intended. For instance, oil-in-water emulsions typically need surfactants with HLB values of 8 to 18 while water-in-oil emulsions require surfactants with HLB values of 4 to 6 (Bouchemal et al. 2004). To prepare an emulsion, the required HLB value of the oil used as the base should match the HLB value of the surfactant. If a blend of surfactants is to be used, the HLB value has to be averaged according to the percentage of surfactants (Americas 1984). Some commonly used surfactants with HLB values between 13 and 17 are polysorbate 60, polysorbate 80, and polysorbate 20. These surfactants are suitable to be used with isopropyl myristate, glycerol monostearate, stearic acid, and oleic acid. As for surfactants with lower HLB values such as lecithin and sorbitan laurate, they are more suitable to be used with hard fats such as cocoa butter (Americas 1984).

The ionic properties of surfactants are also important to be considered during formulation. Anionic surfactants, such as sodium dodecyl sulfate, increase the negative charge of stearylamine-based NLCs to prevent aggregation of these nanoparticles (Lin and Duh 2016). Sodium dodecyl sarcosinate and sodium deoxycholate have also been used in the formulation of electrostatically stable itraconazole–loaded NLCs (Bhadra et al. 2017). As for cationic surfactants, the DL-pyrrolidonecarboxylic acid salt of L-cocoyl arginine ethyl (CAE) ester is a biodegradable surfactant, intended to produce positively charged nanoparticles to target negatively charged cell surfaces (Patil-Gadhe and Pokharkar 2014). In cancer therapy, cationic surfactants such as cetylpyridinium chloride are used to target negatively charged tumor environments (Taveira et al. 2012).

Amphoteric surfactants, also called zwitterionic surfactants, have both anionic and cationic functional groups, thus exhibiting different ionic properties at different pH values. The most commonly used amphoteric surfactants are soybean lecithin and egg lecithin (Zhao et al. 2010; Loo et al. 2013; Han et al. 2014). Non-ionic surfactants remain the most widely used surfactant type (Gordillo-Galeano and Mora-Huertas 2018). Poloxamer 188 that makes up approximately 30% of usage in SLN and NLC formulations has been employed by Velmurugan and colleagues in the formulation of ifosfamide-loaded NLCs (Velmurugan and Selvamuthukumar 2015; Gordillo-Galeano and Mora-Huertas 2018). Tween 80, the most widely used surfactant, has also been used in several NLC formulations for

Table 1 Solid and liquid lipids used for different NLC applications

Component	Types of lipid	Chemical name	Drug(s)	Ref.
Solid lipids	Stearic acid and stearic acid derivatives	Stearic acid	Flurbiprofen	Gonzalez-Mira et al. 2010
		Stearic acid	Tretinoin	Ghate et al. 2016
		Glyceryl monostearate	Simvastatin	Tiwari and Pathak 2011
		Glycerol monostearate	Amphotericin B	Senna et al. 2018
		Glycerol monostearate	Turmeric	Park et al. 2018
		Tristearin	β-Carotene	Oliveira et al. 2016
	Palmitic acid and palmitic acid derivatives	Cetyl palmitate	Resveratrol	Rajput et al. 2018
		Glyceryl tripalmitate	Olanzapine	Jawahar et al. 2018
	Lauric acid derivative	Glyceryl dilaurate	Celecoxib	Joshi and Patravale 2008
	Behenic acid derivatives	Compritol® 888 ATO	Irinotecan	Negi, Jaggi, and Talegaonkar 2013
		Compritol® 888 ATO	Cisplatin, paclitaxel	Yang, Ju, and Dong 2016
	Myristic acid derivative	Trimyristate	Nisoldipine	Dudhipala, Janga, and Gorre 2018
	Fats	Witepsol® E85	Methotrexate	Ferreira et al. 2015
		Softisan® 154	Buparvaquone	Monteiro et al. 2017
		Illipe butter	Thymol	Pivetta et al. 2018
	Combination of different solid lipids	Compritol® 888 ATO/stearic acid	Fluconazole	Kelidari et al. 2017a
		Precirol® ATO5	Bicalutamide	Kudarha et al. 2015
		Precirol® ATO5	LL-37	Garcia-Orue et al. 2016
		Propolis wax/glyceryl behenate	β-Sitosterol	Soleimanian et al. 2018
Liquid lipids	Fatty acids	Oleic acid	Simvastatin	Tiwari and Pathak 2011
		Oleic acid	Bicalutamide	Kudarha et al. 2015
		Oleic acid	Ifosfamide	Velmurugan and Selvamuthukumar 2015
		Oleic acid	Tretinoin	Ghate et al. 2016
		Oleic acid	Fluconazole	Kelidari et al. 2017a
		Oleic acid	Nisoldipine	Dudhipala, Janga, and Gorre 2018
	Monoglycerides and diglycerides	Capmul® MCM	Celecoxib	Joshi and Patravale 2008
		Capmul® MCM	Irinotecan	Negi, Jaggi, and Talegaonkar 2013
		Capmul® MCM	Resveratrol	Rajput et al. 2018
	Triglycerides	Migylol® 812	Methotrexate	Ferreira et al. 2015
		Miglyol® 812	LL-37	Garcia-Orue et al. 2016
		Miglyol® 812	Buparvaquone	Monteiro et al. 2017
		Miglyol® 812	Amphotericin B	Senna et al. 2018
		Medium- chain triglyceride oil	Turmeric	Park et al. 2018
	Other oils	Castor oil	Flurbiprofen	Gonzalez-Mira et al. 2010
		Castor oil	Olanzapine	Jawahar et al. 2018
		High oleic sunflower oil	β-Carotene	Oliveira et al. 2016
		Pomegranate seed oil	β-Sitosterol	Soleimanian et al. 2018
		Calendula oil	Thymol	Pivetta et al. 2018
		Soybean oil	Triamcinolone acetonide	Kraisit and Sarisuta 2018
	Combination of oils	Olive oil, Cremophor® ELP	Cisplatin, paclitaxel	Yang, Ju, and Dong 2016

transdermal and topical delivery (Kumar et al. 2013; Ustundag-Okur et al. 2014; Ghate et al. 2016).

Regardless of the significance of surfactants in nanoparticle formulations, it is crucial to note that their usage in excess can be toxic to the biological system (Lewis 1990). For example, studies show that anionic, cationic, and non-ionic surfactants induce concentration-dependent (0.1 to 1 μg/ml) developmental retardation on zebrafish embryos (Wang et al. 2015). In addition, skin irritant potency of all three types of surfactants at 3% concentration in oil-in-water emulsions was demonstrated using cell viability test and quantification of inflammatory markers in reconstructed human epidermis tissues (Lémery et al. 2015). Poor solubility of non-ionic surfactants with long-chain fatty acids and hydrophobic derivatives provided a false assumption that non-ionic surfactants are less toxic than all other ionic surfactants (Effendy and Maibach 1995). Lemery and colleagues were able to show that a certain non-ionic surfactant such as Beheneth-25 is a stronger irritant than ionic surfactants such as sodium lauryl sulfate and cetyl trimethyl ammonium chloride. Instead, they deduced that the toxicity of surfactants depends on their chemical structure and solubility in water (Lémery et al. 2015).

Selection of methods

The preparation of NLCs can be divided into three types which are high-energy, low-energy, and organic solvent–based preparation (Gordillo-Galeano and Mora-Huertas 2018). High-energy preparation includes the usage of a homogenizer, sonicator, or microwave that employs high energy to reduce the particle size of the formulations. As for low-energy methods, microemulsion and double emulsion are simple methods that do not require the usage of sophisticated equipment to prepare the nanoparticles. Organic solvent–based preparation methods to blend the drug-lipid mixture into a homogenous state are applied in solvent emulsion and diffusion/evaporation and solvent injection. These protocols are described in following sections.

High-pressure homogenization

High-pressure homogenization makes up approximately 50% of SLN and NLC preparation methods (Gordillo-Galeano and Mora-Huertas 2018). It is a technique that employs exertion of high pressure to agitate particles and produce nanostructured samples. In hot high-pressure homogenization, the lipid mixture is first heated and melted at a temperature of 5–10 °C above the melting point of the lipid, before the addition of the drug. A hot surfactant solution that is heated at the same temperature is then added to the lipid mixture, before being subjected to a high shear homogenizer. Subsequently, the pre-emulsion is homogenized again using high pressure at the same temperature. The mixture is then left to cool to room temperature to allow the lipids to form a solid matrix surrounding the drug particle (Üner 2006; Lasoń et al. 2018). Often, increased homogenizer cycles can cause particle aggregation and a higher polydispersity index. However, increased pressure can decrease the particle size (Severino et al. 2012a).

In cold high-pressure homogenization, the lipids are also first melted at 5–10 °C above their melting point, and the drug is added to the lipid melt. The drug-lipid melt is then immediately cooled using dry ice or liquid nitrogen before being milled to micro size. Following that, the solid lipid microparticles are added to the chilled surfactant solution and homogenized at or below room temperature. This technique is employed for hydrophilic drugs and drugs prone to degradation under thermal pressure (Wissing, Kayser, and Muller 2004). However, this method formulates samples with large particle size and a broader particle size distribution (Mehnert and Mader 2001; Shidhaye et al. 2008). Zadeh and colleagues used the cold homogenization technique to encapsulate propranolol hydrochloride, a hydrophilic drug (Zadeh et al. 2018).

Melt emulsification and ultrasonication

In this process, the drug-lipid combo is first melted and blended with a pre-heated surfactant solution at the same temperature. The mixture is then stirred using a magnetic or mechanical stirrer before being sonicated with a probe sonicator. The resulting solution is then immediately cooled to allow the formation of nanoparticles (Yuan et al. 2007; Li et al. 2011). The advantage of this method is the lack of any need for solvents. A probe sonicator is preferred over a bath sonicator due to the higher intensity involved in agitating the samples. However, the use of the probe sonicator can cause metal contamination of the sample, depending on the sonication duration, the probe age, and the mixing protocol (Betts et al. 2013). Moreover, sonication duration

should be optimized to obtain the ideal particle size without overheating the sample. Negi and co-workers found that increased sonication duration reduced particle size and entrapment efficiency (Negi, Jaggi, and Talegaonkar 2013). Excessive acoustic cavitation may disturb the integrity of the lipid matrix and expel the drugs to the aqueous solution, thus resulting in lower particle size (Khan et al. 2016).

Microemulsion

The microemulsion is a technique that was extensively used in the 1990s. Similar to other methods, the initial steps constitute melting and blending the lipid/drug combo before dispersing it into the hot aqueous solution containing surfactant and co-surfactant. The mixture is then agitated to form an initial microemulsion that is thermodynamically stable. This microemulsion is subsequently dispersed in cold water (2–10 °C) to allow rapid recrystallization of oil droplets to form nanoemulsions (Joshi and Patravale 2008; Woo et al. 2014; Ghate et al. 2016). The excess water is then removed via ultrafiltration or lyophilization to form a concentrated dispersion. This is a quick process but, on the downside, there is a need for high concentrations of surfactants and co-surfactants and this is not recommended for regulatory purposes. Furthermore, the process of water removal via ultrafiltration is a difficult task due to the nanoparticle's small size (Wissing, Kayser, and Muller 2004). Removal of water via lyophilization also can modify the physical characteristics of the nanoparticles, in addition to being time-consuming (Beloqui et al. 2016).

Solvent emulsion and evaporation/diffusion

Solvent emulsion-evaporation is a method that uses water-immiscible organic solvents for formulation. Without the need to melt the drug/lipid, organic solvents are used to dissolve and blend the drug/lipid combo, which is then emulsified with the aqueous phase using a high-speed homogenizer. To enhance emulsification, the sample can be passed through a microfluidizer. Then, the organic solvent is evaporated by stirring on a magnetic stirring plate or through a rotary evaporator (Ranpise, Korabu, and Ghodake 2014; Gao et al. 2017). In contrast, solvent emulsion-diffusion uses partially water-miscible organic solvents, to form a thermodynamic equilibrium mixture of organic solvent and water. The water-saturated organic solvent containing drug and lipids (organic phase) is then poured into solvent-saturated water containing the stabilizer (dispersed phase) under continuous stirring to allow the formation of lipid nanoparticles (Hu et al. 2005; Hejri et al. 2013). These solvent-based methods are advantageous for thermo-labile drugs as no heat is required to blend the drug and lipid. The drawback of these systems is the use of organic solvents which may initiate reactions between solvent and drug, thus reducing the drug load of the vehicle (Kaur et al. 2015).

Solvent injection

In this method, the lipid is first dissolved in a solvent that quickly distributes in water, such as dimethyl sulfoxide or ethanol. Once dissolved, the lipid-solvent mixture is injected rapidly into a surfactant solution using an injection needle. The solvent distributes into the aqueous solution while the lipid particles precipitate in the solution. These precipitates are then filtered to obtain the nanoparticles. To ensure a small particle size, the velocity of solvent migration should be high. Hence, less lipophilic solvents are preferred. The advantages of this method are the avoidance of high heat, shear stress, and sophisticated equipment. Nevertheless, the presence of an organic solvent is still undesirable (Schubert and Müller-Goymann 2003; Kaur et al. 2015).

Design of experiments in response surface methodology (RSM)

In the process of optimizing nanoparticle-based formulations, it is important to design the experiments rationally to avoid unnecessary repetition and errors. Design of experiments, often requiring intense statistical considerations, should meet the criteria to economically maximize valid and objective information (Park 2007). During formulations, many factors such as lipid content, drug percentage, surfactant concentration, and instrumental parameters affect the desired properties of the NLCs. Independently optimizing each factor does not only impact efficiency and resources but also ignores the possibilities of multi-response experimental results caused by these factors. To simplify the process of optimization, several first-order and second-order statistical designs have been employed by researchers over the years. First-order designs analyze linear effects of

multiple factors on the responses until a lack-of-fit is noticed in the model. A common first-order design is the 2^k factorial design. Once a curvature is detected, second-order designs are employed by adding several additional points to the model. By performing a reasonable number of experiments from the generated model, a mathematical description of the system behavior is obtained. These descriptions or specifically equations are then used to predict the optimal NLC formulation (Khuri and Mukhopadhyay 2010; Hibbert 2012). Optimization is usually initiated using first-order to screen and narrow down values closest to the optimum value before conducting second-order designs to analytically determine the optimal value (Draper and Hunter 1966). Figure 2 depicts the design of experiments commonly used in NLC formulations.

Plackett-Burman design

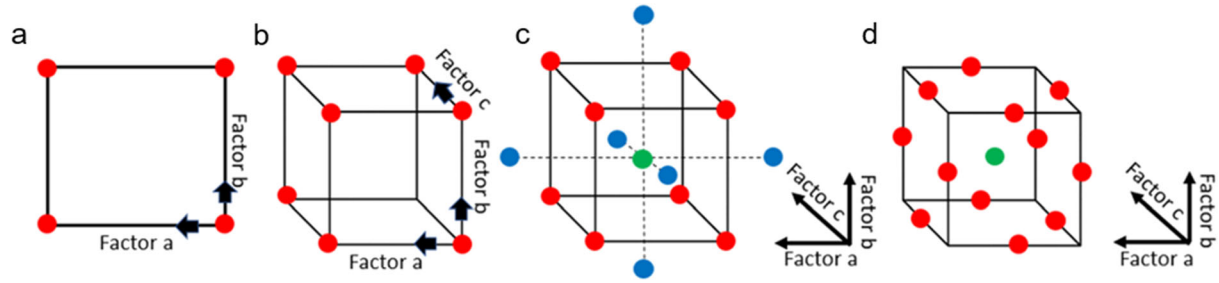
Plackett-Burman is a screening design that evaluates and screens out irrelevant experimental factors with a minimum amount of formulations (Plackett and Burman 1946). Negi and co-workers applied this design to narrow down 11 variables into just 4 factors to be optimized in follow-up experiments (Negi, Jaggi, and Talegaonkar 2013). This is important to efficiently eliminate factors that do not affect the responses significantly. Similarly, Alam and colleagues found that utilizing the Box-Behnken design without Plackett-Burman generated 120 proposed trials, but with Plackett-Burman and Box-Behnken designs, just 49 trials were required to optimize the nanoparticle (Alam et al. 2018). However, the Plackett-Burman design does not give information on the effects of one factor on another. Also, the design does not consider the multiple levels of each factor when implemented in trial runs. Thus, when comparing the joint effects of a few independent factors, this design is not suitable. It is usually used before a general full factorial design, to filter the distinct factors and settle on those that are deemed to affect the responses substantially.

2^k factorial design

In this factorial design, k refers to the number of variables in question, and the base 2 denotes two different levels of the variable, coded as − 1 and 1, also known as low and high levels. 2^k factorial design combines the high and low levels of every variable in the formulation. The number of formulations depends on the number of variables, with two variables resulting in 4 ($2^k = 2^2$) formulations, and three variables resulting in 8 ($2^k = 2^3$) formulations. This design is used for $k < 5$ because, for values larger than 5, the design becomes more intricate with numerous trials (Montgomery 2017). It can also be labeled as a screening design to narrow down important factors for formulation. Tiwari and colleagues used the 2^3 factorial design to determine the effects of three independent variables—amounts of glycerol monostearate, oleic acid, and Poloxamer 407—on the particle size, polydispersity index, zeta potential, entrapment efficiency, and cumulative release of the drug after 55 h (Fig. 3) (Tiwari and Pathak 2011). As only particle size, entrapment efficiency, and percentage of cumulative release were significantly affected by the variables, the polynomial response equations were used to predict the most optimized formulation. The evaluated experimental nanoparticle demonstrated values close to the predicted parameters, thus validating the predictive model (Tiwari and Pathak 2011).

Central composite design

Central composite design (CCD) is perhaps the most utilized of all second-order designs. It consists of 3 portions which are the factorial portion (2^k), the axial portion ($2k$), and the center point replications (n_0). Therefore, the total number of designs is $n = 2^k + 2k + n_0$. Factorial points, axial points, and center points make up the vertices, the symmetrical axial points from the center, and the central point of the design space. These are depicted in Fig. 2c. CCD is an expansion of the 2^k model to build a second-order polynomial for the responses, thus adding experiments that increase optimization conditions. However, CCD is a compacted alternative to the 3^k full factorial design as few experiments are needed for this design (Box and Wilson 1992). Optimization of econazole nitrate (EN)–loaded NLCs was conducted using CCD by determining the particle size and entrapment efficiency of the formulations (Keshri and Pathak 2013). Zhang and colleagues too applied CCD to determine the effects of total solid lipid, liquid lipid concentration, and surfactant concentration on the mean particle size, polydispersity index, zeta potential, and encapsulation efficiency of genistein-loaded NLCs (Zhang et al. 2013a). The generated mathematical model was able to predict a reliable optimized



e

	a	b	c	d	e	f	g
1	+	-	-	+	-	+	+
2	+	+	-	-	+	-	+
3	+	+	+	-	-	+	-
4	-	+	+	+	-	-	+
5	+	-	+	+	+	-	-
6	-	+	-	+	+	+	-
7	-	-	+	-	+	+	+
8	-	-	-	-	-	-	-

f

	a	b	c	d
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	2	1	2	3
5	2	2	3	1
6	2	3	1	2
7	3	1	3	2
8	3	2	1	3
9	3	3	2	1

Fig. 2 Schematic representation of the most commonly used design of experiments in nanostructured lipid carrier formulations. **a** 2^2 factorial design. **b** 2^3 factorial design. **c** Central composite design. **d** Box-Behnken design. For **a** to **d**, red, blue, and green points represent factorial, axial, and center points respectively. The direction of the arrows denotes an increase in the levels of the factors from low to high. **e** Plackett-Burman design. The letters a–g (yellow) represent experimental factors and the numbers 1–8 (green) represent experimental runs. **f** Taguchi orthogonal array. The letters a–d (blue) represent experimental factors and the numbers 1–9 (orange) represent experimental runs. The numbers 1–3 (white) below the factors represent the different levels of the factors

formulation with low percentage bias (Zhang et al. 2013a).

3^k factorial design

The 3^k factorial design, also called the three-level factorial design, is one that applies three levels of each factor, − 1, 0, and + 1. This is a more detailed version of the 2^k full factorial design, where the possible curvature in the response function is taken into consideration. Having another level for a continuous factor enables researchers to study the quadratic relationship between the response and each factor. However, due to budget and time constraints, the 2^k full factorial design with center points (CCD) is preferred over 3^k full factorial design, as it too can identify the presence of curvature. The 3^k factorial design can be further classified into 2 types, which are Box-Behnken designs and 3^{k-p} orthogonal arrays (Montgomery 2017). Several other factorial designs have been used by other researchers in the formulation of NLCs. These are detailed in Table 2.

Box-Behnken design The Box-Behnken design (BBD) employs 3 levels of each factor, which differs from CCD that can have up to 5 levels of each factor. It does not include the extreme levels of the factors, thus allowing lesser design points than CCD. The design can estimate the first- and second-order coefficients without including runs from a factorial experiment (Box and Behnken 1960). Consequently, BBD is not suitable for sequential experiments. Kudarha and colleagues used this design to optimize bicalutamide-loaded NLCs by designating total lipid percentage, liquid lipid percentage, and soya lecithin percentage as the variables and particle size and entrapment efficiency as the responses (Kudarha et al. 2015). In addition, methotrexate-loaded NLCs were optimized using BBD with the amount of liquid lipid, surfactant, and drug as the independent variables and particle size, polydispersity index, and entrapment efficiency as the dependent variables. The experimental values of the responses were close in proximity to the predicted values, hence confirming the robustness of the design (Ferreira et al. 2015).

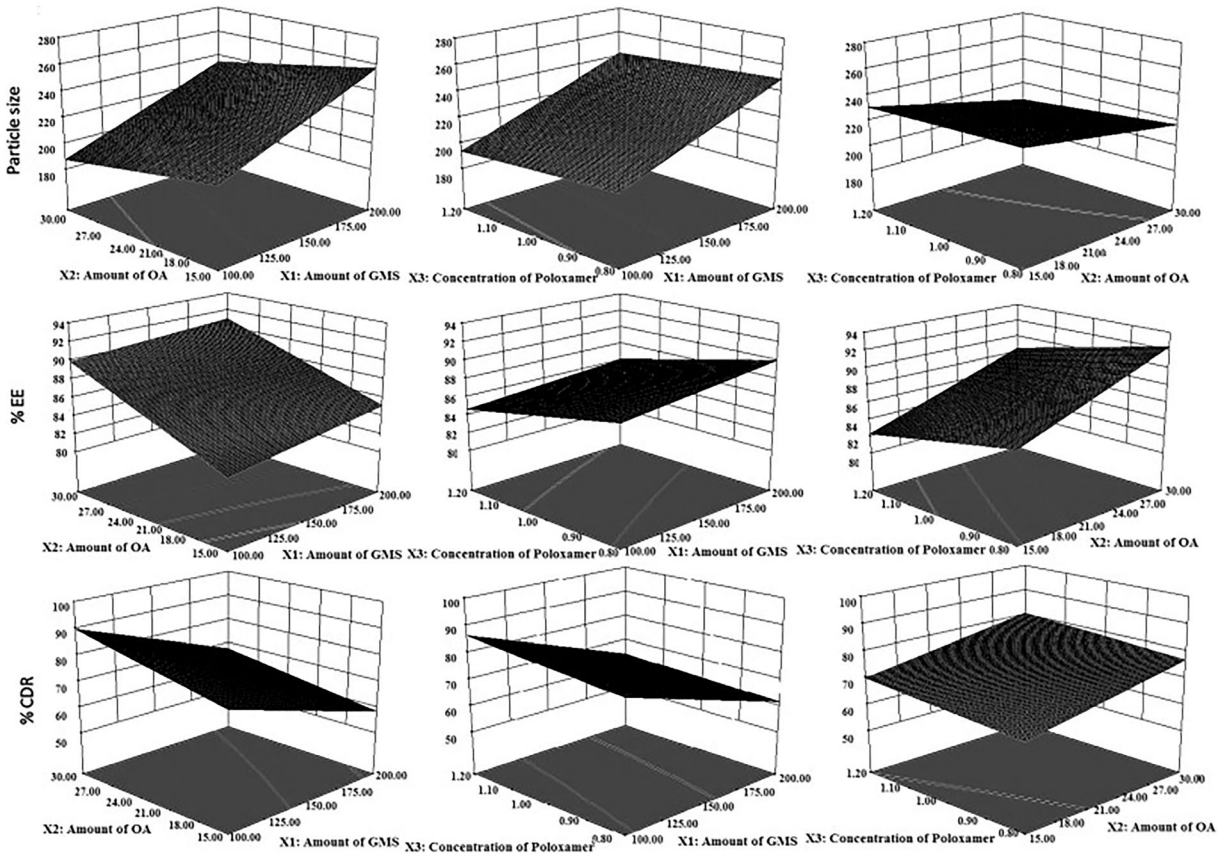


Fig. 3 RSM plots showing multi-response situations when the independent variables (amount of oleic acid, amount of glycerol monostearate, and concentration of Poloxamer) are simultaneously manipulated to determine the responses (particle size, entrapment efficiency, and percentage of cumulative drug release) in the optimization of simvastatin-loaded nanostructured lipid carrier (Tiwari and Pathak 2011)

3^{k-p} orthogonal array A 3^{k-p} orthogonal array is a type of fractional design that is also used to screen linear and quadratic effects in the initial or middle stage of formulations. This is usually followed by a full factorial design after screening. Also known as the Taguchi design, the array predicts experimental conditions with the least variability (Taguchi 1960). In the formulation of blank NLCs, the L9 Taguchi orthogonal array was employed on three types of surfactants and total lipid concentration at three different levels to optimize the particle size (Negi, Jaggi, and Talegaonkar 2013). The experimental conditions with the highest signal-to-noise ratio were deemed as the most optimal conditions. Only 9 experimental runs were required to identify the types of surfactant and total lipid concentration for the most optimal particle size and zeta potential for blank NLCs (Negi, Jaggi, and Talegaonkar 2013). Emami and colleagues also applied the L8 orthogonal array to determine the particle size, polydispersity index, zeta potential, entrapment efficiency, drug load, and drug release rate (Emami et al. 2012). The experimental values for the most significantly affected responses corresponded with the predicted values with negligible error percentage, thus validating the method (Emami et al. 2012).

Desired characteristics and associated variables

Particle size

Particle size is an essential parameter to consider in the process of optimizing NLCs. Dynamic light scattering (DLS) is the most common method used to determine particle size distribution. Ideally, particle size between 50 and 200 nm is preferred for drug delivery to biological cells because particles less than 10 nm will be cleared by the kidneys, whereas particles greater than 200 nm will be recognized by the mononuclear

Table 2 Studies that use response surface methodology (RSM) to optimize NLC formulations

RSM design	Study	Experimental variables	Measurable responses	Reference
Plackett-Burman	Irinotecan-loaded NLC for chemotherapy	Temperature (°C) Drug to lipid percentage (%) Needle size (gauge) Stirring speed (RPM) Organic to aqueous phase ratio Lecithin percentage (%) Pluronic F-68 percentage (%) Sodium deoxycholate percentage (%) Lipid content (%) Injection speed (ml/min) Sonication time (s)	Particle size (nm) Zeta potential (mV) Entrapment efficiency (%)	Negi et al. 2013
	Isradipine-loaded NLC for the treatment of hypertension	Total lipid percentage (% w/v) Solid lipid to liquid lipid ratio Surfactant (% w/v) Surfactant to co-surfactant ratio Stirring speed (rpm) Sonication time (min) Temperature (°C) Amount of drug (mg)	Particle size (nm) Entrapment efficiency (%) Cumulative drug release at the 24th hour (%)	Alam et al. 2018
2^k full factorial	Flurbiprofen-loaded NLC for ophthalmic medication	Solid lipid to total lipid percentage (%) Drug concentration (%) Stabilizer concentration (%) Storage temperature (°C)	Particle size (nm) Polydispersity index Zeta potential (mV)	Gonzalez-Mira et al. 2010
	Simvastatin-loaded NLC for biodistribution study	Amount of solid lipid (mg) Amount of liquid lipid (mg) Surfactant concentration (%)	Particle size (nm) Entrapment efficiency (%) Cumulative drug release at the 55th hour (%)	Tiwari and Pathak 2011
	Effect of homogenization technique on NLC and SLN	High-shear homogenization intensity (rpm) High-pressure homogenization pressure (bar)	Particle size (nm) Polydispersity index Zeta potential (mV)	Severino et al. 2012a
	Rosuvastatin-loaded NLC for pulmonary targeting	Lipid concentration (%) Drug concentration (%) Surfactant concentration (%)	Particle size (nm) Entrapment efficiency (%)	Patil-Gadhe and Pokharkar 2016
Central composite	β-Carotene-loaded NLC to increase the bioavailability of the compound	Liquid lipid to total lipid ratio Lipid phase concentration (%) Surfactant concentration (%) Aqueous phase temperature (°C)	Particle size (nm) β-Carotene retention (%)	Hejri et al. 2013
	Genistein-loaded NLC for the prevention of posterior capsular opacification	Drug concentration (%) Gelucire 44/14 concentration (%) Liquid lipid concentration (%) Surfactant concentration (%)	Particle size (nm) Polydispersity index Zeta potential (mV) Entrapment efficiency (%)	Zhang et al. 2013a
	Ifosfamide-loaded NLC for oral delivery in cancer therapy	Drug to lipid ratio Organic to aqueous phase ratio Surfactant concentration (%)	Entrapment efficiency (%) Drug loading efficiency (%) Particle size (nm)	Velmurugan and Selvamuthukumar 2015
	Buparvaquone-loaded NLC for leishmaniases	Solid to liquid lipid ratio Surfactants concentration (%)	Particle size (nm) Polydispersity index	Monteiro et al. 2017
	β-Sitosterol-loaded NLC for food applications	Percentage of the drug (%) Percentage of liquid lipid (%) Type of solid lipids	Particle size (nm) Polydispersity index Zeta potential (mV) Entrapment efficiency (%)	Soleimanian et al. 2018
Box-Behnken	Methotrexate-loaded NLC for cancer therapy	Amount of liquid lipid (mg) Amount of surfactant (mg) Amount of drug (mg)	Particle size (nm) Polydispersity index Entrapment efficiency (%)	Ferreira et al. 2015

Table 2 (continued)

RSM design	Study	Experimental variables	Measurable responses	Reference
	Silymarin-loaded NLC to improve the lymphatic transport pathway	Solid to liquid lipid ratio Surfactant concentration (%) Homogenization speed (rpm)	Particle size (nm) Entrapment efficiency (%)	Chaudhary et al. 2015
	Bicalutamide-loaded NLC for cancer therapy	Amount of total lipid (g) Amount of liquid lipid (%) Soya lecithin concentration (%)	Particle size (nm) Entrapment efficiency (%)	Kudarha et al. 2015

phagocyte system (MPS) (Maeda et al. 2000; Torchilin 2011; Li et al. 2012). Due to these reasons, particle size serves as an important feature for NLCs. Variables such as surfactant concentrations, drug-to-lipid ratio, liquid-lipid percentage, and sonication time affect particle size significantly. These variables will be analyzed to examine their effects during optimization by determining a positive or negative correlation with the nanoparticle characteristics.

Type of lipids

One of the factors that affect particle size is the melting point of solid lipids. Lipids of higher melting point result in larger particle size due to the higher melt viscosity present in the system. In the formulation of β-sitosterol-loaded NLC, formulations made of propolis wax resulted in particles of smaller size compared with formulations made with propolis wax and glyceryl behenate, due to glyceryl behenate's high melting point. Similarly, Compritol® 888 ATO was found to result in NLCs of bigger particle size compared with lipids of low melting points (Lasoń, Sikora, and Ogonowski 2013). Besides that, solid lipids of higher molecular weight caused the formation of particles with bigger sizes due to increased surface tension. This was demonstrated in the comparative studies of cholestryl stearate (653.12 g/mol) and cholesterol (386.7 g/mol) (Andalib et al. 2012). Another trait that affects particle size is the lipid's emulsifying property. In the formulation of amisulpride-loaded NLCs, Gelucire® 43/01 produced particles of smaller size compared to tripalmitin, due to its emulsifying property. This trend was also noticed in other studies involving Gelucire® 43/01 and Gelucire® 50/13 (Date et al. 2011; Fatouh et al. 2017). As for the type of liquid lipid, lipids with more unsaturation in their chemical structure produce particles of smaller size due to their ability to be compacted easily. This was observed in the formulations of 5-FU-loaded NLCs using oleic acid which has unsaturation in its structure, compared to octanol that has a linear structure (Andalib et al. 2012).

Type of surfactants

In terms of surfactant types, non-ionic surfactants were found to produce particles of smaller size compared to anionic, cationic, and amphoteric surfactants, in the formulation of itraconazole-loaded NLCs (Bhadra et al. 2017). Associative interaction with resultant tight molecular packing is seen between non-ionic surfactants and lipid components, thus the smaller particle size (Ghorab, Gardouh, and Gad 2015; Nahak et al. 2015). In addition, the number of surfactants negatively correlates with the particle size of NLCs. Karn-Orachai and colleagues found that the average particle size of NLCs made of non-ionic, anionic, and cationic surfactants exhibited the smallest particle size, in comparison to NLCs made up of only one or two of those surfactants (Karn-Orachai et al. 2014). This result was supported by the explanation that Coulombic interactions between surfactants of similar charges led to particles of larger size. Similar results from a previous report investigating the effects of surfactant types on drug-free NLCs were able to validate this trend (Han et al. 2008).

Surfactant concentration

In the formulation of methotrexate-loaded NLCs, polyvinyl alcohol (PVA) was used as a surfactant at amounts of 40, 50, and 60 mg. There was a negative correlation between the variables, whereby an increase in surfactant concentration resulted in a decrease in particle size (Ferreira et al. 2015). Ifosfamide-loaded NLCs also demonstrated a negative correlation with the increase in Poloxamer 188 from 0.25 to 1% (Velmurugan and Selvamuthukumar 2015). In addition, the studies involving macrophage delivery of buparvaquone demonstrated that, when Tween 80 and Kolliphor® P188 are

used together, more than 3% surfactant is needed to reach low particle size with a solid-to-liquid lipid ratio of 2.75 and above (Monteiro et al. 2017). Overall, these investigations show that increasing surfactant concentration causes an anticipated decrease in particle size. This condition is caused by the decline in interfacial tension between the lipid and the environmental phase, thus resulting in particle partition (Bnyan et al. 2018). Nonetheless, there is a limit to the amount of surfactant that can reduce the particle size, and once this limit is reached, increasing surfactant concentration will not affect particle size (Harivardhan Reddy et al. 2006; das Neves and Sarmento 2015).

Ratio of solid-to-liquid lipid

NLCs are composed of solid and liquid lipids. This forms liquid globules within the solid matrix, in which the drug molecules are encapsulated. Hence, it is necessary to have the correct ratio of solid-to-liquid lipids. In bicalutamide-loaded NLCs, increasing oleic acid percentage (20–40% out of total lipid) reduced the particle size (Kudarha et al. 2015). Buparvaquone-loaded NLCs also showed less than 220 nm of average particle size when the solid-to-liquid lipid ratio was less than 2.5 units (Monteiro et al. 2017). Furthermore, in β-carotene-loaded NLCs, increasing corn oil percentage (0–40%) with respect to the total lipid caused a slight decrease in particle size (Hejri et al. 2013). In most cases, an increase in liquid lipid percentage reduces the size of the NLCs, but this is only true within certain percentage limits. Liquid lipid moderates the viscosity within the solid matrix and, thus, results in the formation of smaller sized particles (Emami et al. 2012). There are also reports of conflicting results by Woo and colleagues (Woo et al. 2014). Particles with increasing oleic acid composition from 0 to 30% displayed a trend of increasing size, while those with 40–50% oleic acid showed otherwise (Fig. 4a). Transmission electron microscope images demonstrated that the stearic acid and oleic acid merger resulted in rod-shaped particles instead of the assumed spherical shape, which may explain the discrepancy in the particle size (Fig. 4b, c). This rod-shaped by-product is common in stearic acid–based nanoparticles where solvent interaction can also alter their shape (Pizzol et al. 2014). Hence, any measurement of particle size using DLS should be revalidated using electron microscopy, to ensure that the trend predicted based on solid-to-liquid lipid ratio is verified.

Drug concentration

Drug concentration is another factor that affects particle size. Increasing the amount of methotrexate from 0 to 20 mg within the Witepsol®/Miglyol® matrix positively correlated with the particle size (Ferreira et al. 2015). Similarly, the addition of genistein in genistein-loaded NLC (0.05–0.25%) caused an increase in the particle size of the system (Zhang et al. 2013a). Besides, in the formulation of triamcinolone acetonide–loaded NLCs, increasing drug-to-lipid ratio (55–95%) resulted in an increase in the particle size. These outcomes can be attributed to the added incorporation of drug compounds within the lipid matrix or on the NLC surface, consequently enlarging the particles. Furthermore, the addition of the solid phase in the lipid matrix increases the viscosity of the system, hence increasing the overall particle size (Varshosaz et al. 2014). Several other studies have reported similar variations in particle size with modifications in drug percentage (Zhang et al. 2010; Kumbhar and Pokharkar 2013). While in the case of topotecan, a reduction in particle diameter was observed with the addition of active compounds. This was due to topotecan's co-surfactant-like properties which has an overall negative correlation with particle size (Souza et al. 2011).

Polydispersity index

The polydispersity index (PDI) is a measure of the dispersion quality of the particles, indicating, whether they are monodispersed or polydispersed. A PDI value of less than 0.3 is considered ideal in nanoparticle formulation to ensure homogeneity and to prevent aggregation (Zhang, Fan, and Smith 2009; Das and Chaudhury 2011). Types of surfactants and surfactant concentration affect the PDI of NLCs.

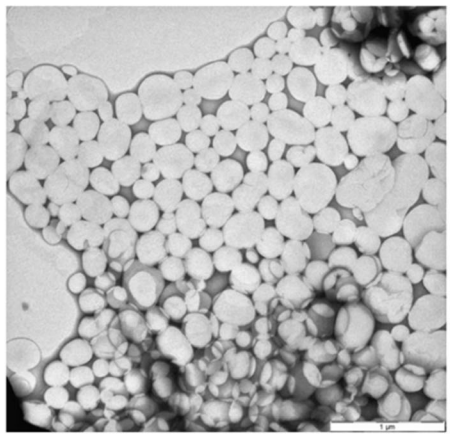
Type of surfactants

The ionic properties of surfactants affect PDI of NLCs. Non-ionic surfactants resulted in decreased PDI values compared to anionic, cationic, and amphiphilic surfactants in the formulation of drug-free NLCs (Bhadra et al. 2017). This is in accordance with their ability to lower the particle size and reduce the aggregation tendency. Rathod and co-workers also found that non-ionic surfactants exhibited nanoparticle systems with reduced PDI values compared with other surfactants.

a

OA ratio (wt%)	Unloaded SONs			Salicylic acid loaded SONs		
	Mean particle size (nm)	Polydispersity index	Zeta potential (mV)	Mean particle size (nm)	Polydispersity index	Zeta potential (mV)
0	194 ± 2	0.19 ± 0.01	−45.9 ± 0.9	271 ± 3	0.25 ± 0.01	−39.4 ± 0.5
10	221 ± 4	0.21 ± 0.02	−47 ± 2	283 ± 8	0.29 ± 0.03	−43 ± 1
20	246 ± 3	0.31 ± 0.03	−45.5 ± 0.5	311 ± 7	0.35 ± 0.02	−45 ± 1
30	255 ± 3	0.37 ± 0.02	−50 ± 1	332 ± 3	0.37 ± 0.03	−43 ± 1
40	229 ± 4	0.37 ± 0.01	−43 ± 1	330 ± 10	0.40 ± 0.02	−44.0 ± 0.8
50	223 ± 4	0.36 ± 0.02	−46 ± 1	280 ± 5	0.38 ± 0.02	−45.2 ± 0.7

b



c

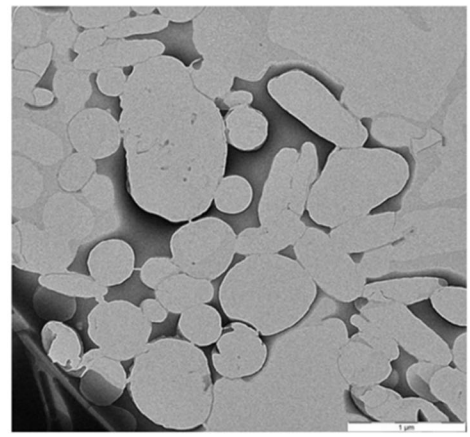


Fig. 4 **a** Mean particle size data of unloaded and loaded SLN/NLC. The mean particle size is seen to increase with an increased oleic acid percentage from 0 to 30%, **b** spherical stearic acid SLN (without any liquid lipid), and **c** rod-shaped stearic acid-oleic acid NLC (with liquid lipid) (Woo et al. 2014)

Kolliphor® HS15 and Kolliphor® P188 with their hydrophilic and hydrophobic moieties confer steric stability to the NLCs in the presence of water and lipids, thus resulting in low PDI (Rathod, Shah, and Dave 2020). Furthermore, the usage of more than one surfactant was reported to reduce the PDI of the dispersion as compared with the usage of only one surfactant (Gonzalez-Mira et al. 2010; Severino et al. 2011). Aggregation of particles is further inhibited when at least two surfactants are present during the stressful formulation procedure (Severino et al. 2011).

Surfactant concentration

In most cases, an increase in surfactant concentration leads to higher PDI due to particle aggregation caused by surfactants bound to the surface (das Neves and Sarmento 2015; Ferreira et al. 2015). Figure 5a shows that the PDI of methotrexate-loaded NLCs raised with an increment in PVA concentration (Ferreira et al. 2015). Similarly, flurbiprofen-loaded NLCs demonstrated an increase in PDI with an increase in Tween 80 percentage from 1.6 to 2.6 wt% (Gonzalez-Mira et al. 2010). An identical pattern was also noted with increased surfactant concentration in acetonide-loaded NLCs (Araujo et al. 2010). Zhang and co-workers, however, reported that at higher drug concentrations, the addition of surfactant led to lower polydispersity index (Fig. 5b) (Zhang et al. 2013a). This was supported by an inference that there is a need for surfactants to stabilize the system when increased drug molecules attempt to aggregate the system, thus leading to a significantly lower polydispersity index (Zhang et al. 2013a).

Zeta potential

Zeta potential is the measure of the potential difference across a particle that assesses the aggregation tendency of nanoparticles. Values less than − 30 mV and greater than + 30 mV are considered ideal for the particles to repel each other (Mitri et al. 2011). In the formulation of

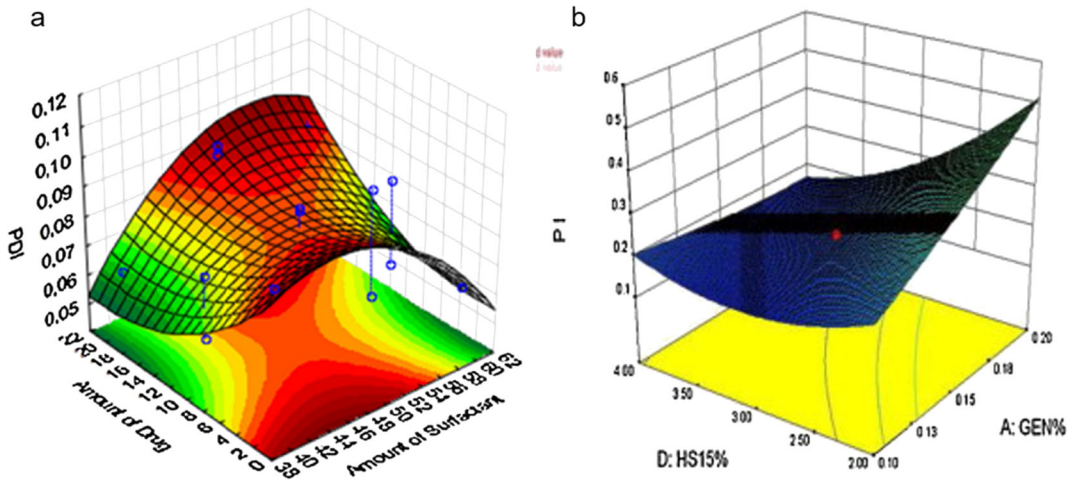


Fig. 5 **a** Surface response chart shows that increased amount of surfactant results in higher polydispersity index until a certain limit is reached (Ferreira et al. 2015). **b** Surface response chart shows that at higher drug (genistein) concentration, increasing surfactant (HS15) concentration leads to lower polydispersity index (Zhang et al. 2013a)

irinotecan-loaded NLCs, the zeta potential of the NLC particles was positively influenced by the drug-to-lipid ratio, organic-to-aqueous phase ratio, and sonication time (Negi, Jaggi, and Talegaonkar 2013). Likewise, increasing drug concentration in flurbiprofen-loaded NLC made the zeta potential less negative, perhaps due to the accumulation of drug molecules on the surface of particles (Fig. 6) (Gonzalez-Mira et al. 2010). The interference of drug molecules on the surface of lipids reduces the negative potential of the particles. Also, increased liquid lipid concentration formed particles with more negative zeta potential as noted by Kelidari and co-workers (Kelidari et al. 2017b). The zeta potential can also be manipulated by changing the type of liquid lipid used. Oleic acid gives a more negative zeta potential compared to octanol due to the presence of the carboxylic acid group in its structure (Andalib et al. 2012). Besides that, the addition of surface modifiers such as chitosan oligosaccharide lactate can shift the zeta potential from negative to positive (Ustundag-Okur et al. 2014). This feature can be applied in the targeted delivery of nanoparticles to negatively charged tumors.

Entrapment efficiency

Entrapment efficiency measures the efficiency of the carrier to encapsulate the drug. It is calculated by determining the percentage of drug incorporated in the carrier (W_T) from the total amount of drug added during the formulation (W_I) (Piacentini 2016). The formula for measuring entrapment efficiency (EE) is as follows:

$$EE = (W_T/W_I) \times 100\%$$

Usually, the amount of drug added corresponds to the drug's solubility in the liquid or solid lipid. Nevertheless, entrapment can be affected by the presence of organic solvents or physical stress during the formulation process, such as when exposed to homogenization and ultrasonication (Yuan et al. 2007; Severino et al. 2012a; Negi et al. 2013). In this section, parameters such as types of lipids and surfactants, surfactant concentration, and drug concentration that significantly affect entrapment efficiency will be discussed.

Types of lipids and surfactants

In the formulation of NLCs, the usage of lipids with complex structures ensures greater entrapment of drugs. It was found that using cholestryl stearate instead of cholesterol increased the entrapment efficiency of 5-FU-loaded NLCs (Andalib et al. 2012). In addition, Soleimanian and colleagues demonstrated that propolis wax and glyceryl behenate mixture for the lipid matrix resulted in higher entrapment efficiency of β-sitosterol compared with propolis wax alone (Soleimanian et al. 2018). Greater distance between fatty acid chains and spaces created by structural imperfections in the lipid core are vital reasons to incorporate more drugs (Asumadu-Mensah, Smith, and Ribeiro 2013). Similar results were seen when Gelucire® 43/01 was used instead of tripalmitin in the formulation of amisulpride-

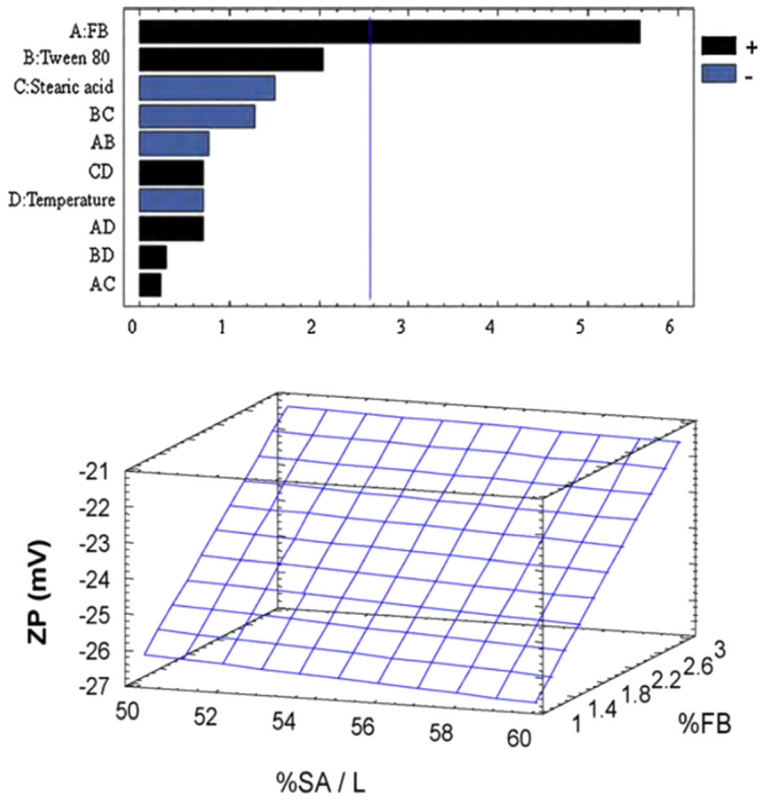


Fig. 6 Pareto chart and surface response chart show that increasing the percentage of flurbiprofen (drug) produces nanoparticles with less negative zeta potential (Gonzalez-Mira et al. 2010)

loaded NLCs (El Assasy et al. 2019). The diverse composition of fatty acids in the Gelucire ensues disorder in the lipidic structure to create voids for active compound accommodation (Tsai et al. 2012). In terms of surfactants, entrapment efficiency decreased in the order of non-ionic, zwitterionic, anionic, and cationic surfactant usage (Bhadra et al. 2017). High steric stabilization of the nanoparticle complex is provided by non-ionic surfactants, enabling harmonization of lipid components and retention of the drug (Ricci et al. 2005).

Surfactant concentration

Kumbhar and co-workers predicted that entrapment efficiency negatively correlates with surfactant concentration (Kumbhar and Pokharkar 2013). Such a case was noticed in the optimization of methotrexate-loaded NLC, whereby increasing PVA concentration caused the entrapment efficiency to decrease (Fig. 7a) (Ferreira et al. 2015). Similar results were seen with the addition of soy lecithin in the formulation of another type of NLC (Kudarha et al. 2015). Ferreira and colleagues inferred that surfactants enabled the partitioning of drugs from the internal phase of the nanoparticle to the external phase. Hence, entrapment efficiency of the NLC is reduced (Ferreira et al. 2015). There should be a maximum limit to the amount of surfactant added because within this limit, increasing surfactant concentration may improve entrapment efficiency. Such a case was seen in the formulation of ifosfamide-loaded NLCs, where higher amounts of Poloxamer 188 and sodium alginate enhanced the entrapment efficiency slightly (Velmurugan and Selvamuthukumar 2015).

Drug concentration

In methotrexate-loaded NLCs, increasing the amount of drug improved the entrapment efficiency, which was seen in both linear and quadratic relationships (Ferreira et al. 2015). On the contrary, Velmurugan and colleagues discovered that increasing drug-to-lipid ratio negatively correlated with the entrapment efficiency (Velmurugan and Selvamuthukumar 2015). To justify both studies, Negi and co-workers reported that an initial increase in entrapment efficiency was noted with increasing drug concentration, but this declined once the

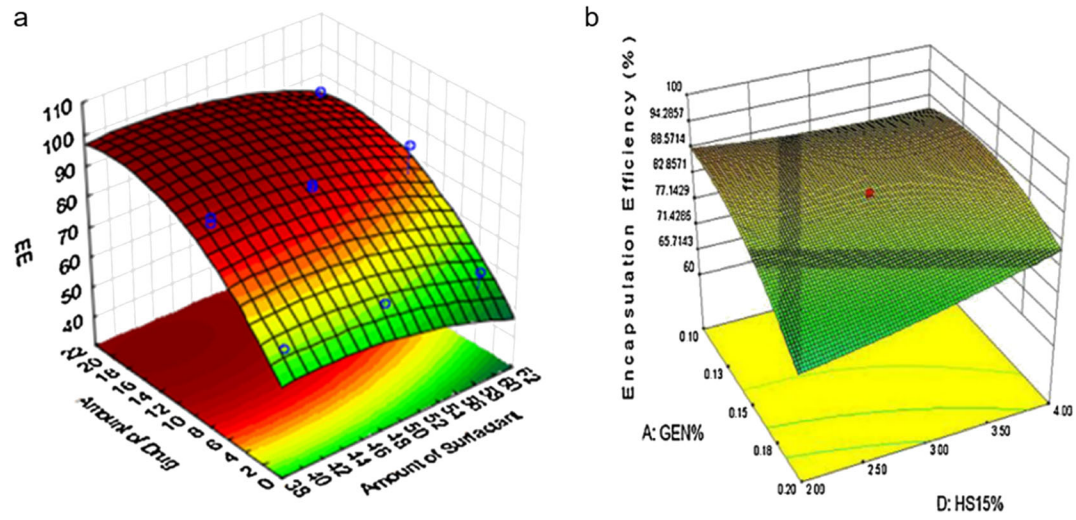


Fig. 7 **a** Surface response chart shows that increasing the amount of surfactant results in lower entrapment efficiency at all concentrations of the drug (Ferreira et al. 2015). **b** Surface response chart shows that addition of drug after a certain limit has a negative correlation on the entrapment efficiency (Zhang et al. 2013a)

lipid had reached its maximum limit of assimilating the drug (Negi, Jaggi, and Talegaonkar 2013). An example of this limit is seen in the preparation of genistein-loaded NLC where the entrapment efficiency reduces after the addition of more than 15 mg of the drug (Fig. 7b) (Zhang et al. 2013a). To obtain an optimized amount of drug load, the drug should be added up to the maximum limit of the lipid matrix. This amount is usually established in a preliminary solubility test involving drugs and lipids (Kasongo et al. 2011).

In vitro drug release

The most common methods for conducting in vitro drug release studies use the dialysis membrane or Franz diffusion cell (D'Souza 2014). In vitro drug release studies examine release kinetics of the drug from the carrier using different mathematical models. For accurate assessments, drug delivery systems should be optimized according to the type of delivery intended and their mechanism of drug release. Immediate or modified release and delayed or extended-release provide the basis for studying in vitro drug release (Arifin, Lee, and Wang 2006; Dash et al. 2010). The immediate-release follows the first-order kinetics profile and is normally used for drugs intended for instant relief, such as in cases of anti-inflammation, anti-allergens, and anti-psychotics (Preetha, Srinivasa, and Pushpalatha 2015). Delayed-release is especially important in the oral delivery of drugs that need to be protected from degradation. To avoid the low pH level of the stomach, the specific drug should be able to reach the intestines and be released there where the environment is more conducive for absorption into the bloodstream. Extended-release is vital in chronic illnesses in which frequent dosing is not preferred. Extended-release can be divided into sustained release and controlled release. Sustained release is the most desired form of release in nanoparticle-based formulations and this release can either follow zero-order kinetics or linear release as a function of the square root of time. Controlled release, on the other hand, requires the concentration of the drug in the body to be regulated, which requires the timed release of the drug from the nanoparticle (Perrie and Rades 2012).

Type of lipids

Solid and liquid lipids of different melting points result in a biphasic drug release pattern in most NLCs, with initial burst release followed by a sustained one. The faster crystallization of solid lipid extrudes the liquid lipid to the carrier surface along with the dissolved drug. This causes immediate release of drug once exposed to an aqueous environment (Elmowafy et al. 2018; Tan et al. 2019). On another case, cholesterol-based NLCs exhibited slower drug release after 20 h compared to cholestryl stearate–based NLCs as a result of hydrogen bond formation between the fluorine group of 5-FU and hydroxyl groups of cholesterol (Andalib et al. 2012). These findings are essential to be studied for

pharmaceutical applications that require immediate relief followed by sustained drug activity to avoid repeated administrations (Preetha, Srinivasa, and Pushpalatha 2015).

Liquid lipid or surfactant concentration

The rate of drug release can also be changed by modifying the amounts of liquid lipid or surfactant. For instance, the initial drug release rate of paclitaxel-loaded NLCs was positively affected by liquid lipid and surfactant concentration (Emami et al. 2012). Simvastatin-loaded NLCs also showed enhanced drug release rate when the liquid lipid or surfactant concentrations were increased (Fig. 8a, b) (Fathi et al. 2018). Similarly, Fig. 8c shows how the cumulative release of paclitaxel is affected by different amounts of liquid lipids (Gordillo-Galeano and Mora-Huertas 2018). In each case, Fickian diffusion flux was the dominant mechanism that controlled the release rate. These results are associated with the decrease in particle size with increased amounts of liquid lipid and surfactants that improves particle surface area for faster release of the content (Fathi et al. 2018). Taking into consideration the types of applications the NLCs are intended for, these different forms of release can be regulated by modifying the amount of components.

Storage stability

Lipid-based nanoparticles are prone to aggregation (Beloqui et al. 2016). To ensure the stability of NLC dispersions over a long period, it is imperative that water content is removed from the suspension. This is also to protect the NLC particles from bacterial contamination. Removal of moisture can be achieved through freeze-drying or spray drying (Obeidat et al. 2010; Varshosaz, Eskandari, and Tabbakhian 2012). In cases where removal of water is not preferred, preservatives can be added for long-term stability.

Freeze-drying or lyophilization is the process of using freezing temperature to dehydrate samples through sublimation. However, the resulting sample may have a wide particle size distribution and undergo aggregation. To overcome this, it is important to add a cryoprotectant that can reduce changes in particle size (Beloqui et al. 2013). Cryoprotectants are usually made up of sugars such as lactose, glucose, or trehalose, and in some cases sugar alcohols, such as mannitol or sorbitol (Emami et al. 2012; Al-Qushawi et al. 2016). Avicel is a type of microcrystalline cellulose that can also be used to protect lipids at extreme temperatures (Varshosaz, Eskandari, and Tabbakhian 2012). The usage of these cryoprotectants for different lipid components is as shown in Table 3.

Spray drying requires the use of hot air to rapidly evaporate water from a sample and to produce free-flowing powder. This is not suitable for samples with low melting points or degradation temperatures. The lipid may partially melt and cause particle growth. It is, however, commonly used in other polymer-based nanoparticles, such as poly(lactic-co-glycolic) acid (PLGA). In NLC studies, paclitaxel-loaded NLCs were spray-dried to avail free-flowing powders for pulmonary delivery to lung cancer cells (Kaur et al. 2016).

Besides removing water, preservatives can be added to enhance the stability of NLCs suspended in water. Obeidat and colleagues investigated the effect of 11 different preservatives on Q10-loaded NLC dispersions and found that Hydrolite-5 was the most effective in preserving the stability of the NLC (Fig. 9) (Obeidat et al. 2010). It is imperative to note that the preservative should be non-ionic so that it negligibly affects the zeta potential, an important criterion in stability. In addition, the preservative should be hydrophilic to prevent affinity towards the lipid surface (Obeidat et al. 2010).

Modifications for targeted delivery

The discussed characteristics, such as small size, low polydispersity, non-zero zeta potential, suitable release kinetics, and high storage stability, are essential for the sustained systemic circulation of an extremely protected drug carrier system. However, targeted delivery of the drug to the selected organ or cell without accumulating in unwanted regions is still a recurring problem. Targeted drug delivery systems, especially those involving intravenous administration, ideally should employ 4 traits, which are, retain, evade, target, and release in the biological environment (Mills and Needham 1999). This means the drug should remain entrapped in the delivery vehicle during the circulation process to reach the targeted site and be released from the vehicle within the timepoint required for its effective function.

Current chemotherapy treatments based on targeted delivery are still far from being perfect at the clinical

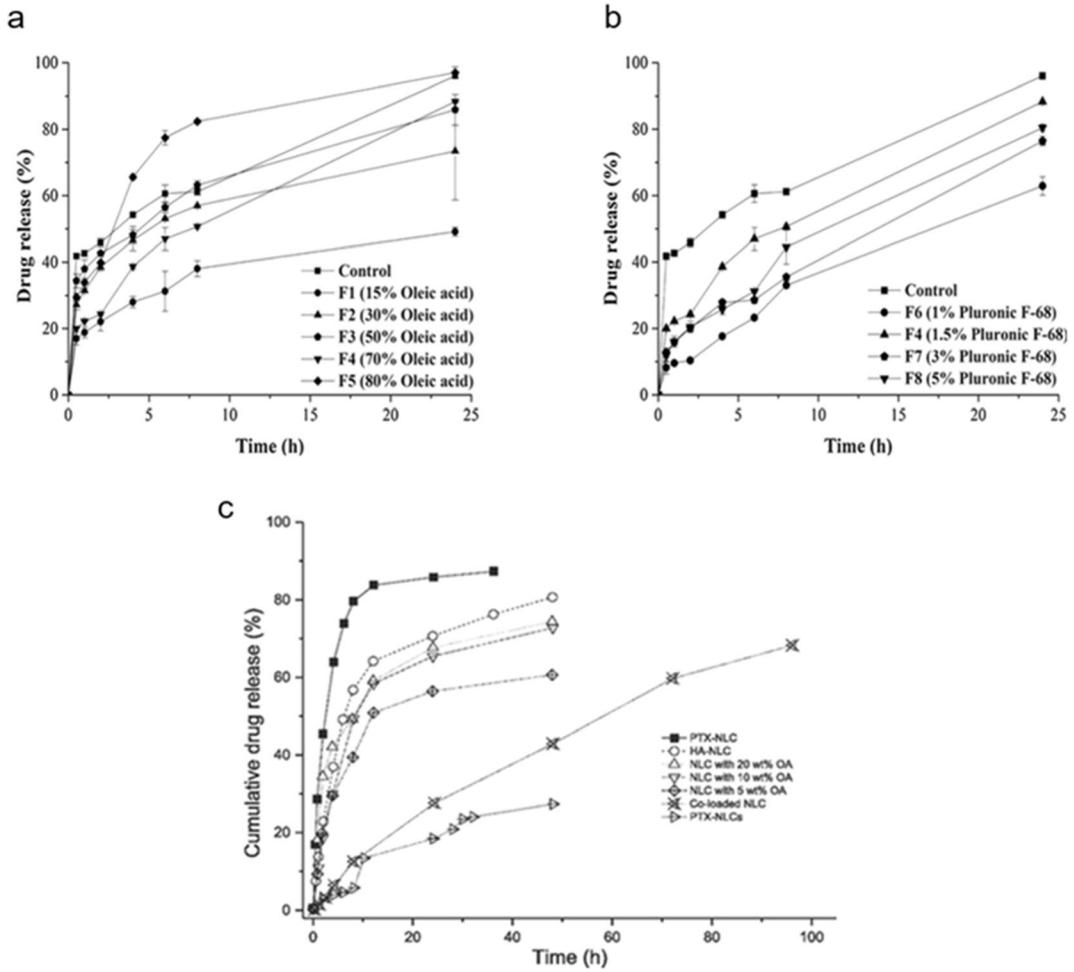


Fig. 8 **a** Drug release profile of simvastatin-loaded NLC with varied oleic acid content shows that increasing oleic acid percentage results in a faster release of the drug. **b** Drug release profile of simvastatin-loaded NLC with different amounts of surfactant (Pluronic F-68) shows that increasing the surfactant concentration results in a faster release of the drug (Fathi et al. 2018). **c** Cumulative drug release of paclitaxel from NLC with different amounts of oleic acid supports the trend seen with increased amounts of liquid lipid and faster rate of release (Gordillo-Galeano and Mora-Huertas 2018)

level. In most cases, nanoparticle-based formulations for intravenous administration are prepared with dependence on blood circulation and extravasation (Torchilin 2000). Therefore, the accumulation of the drug-loaded nanoparticle is not 100% at the targeted site but can be distributed in unintended organs. Targeting can be active or passive, with active targeting preferred over passive targeting that depends on enhanced permeation and retention (EPR) effect (Bae and Park 2011) (Fig. 10). Active targeting depends on ligand-receptor interaction which requires the ligand and receptor to be at a distance of < 0.5 nm from each other (Béduneau et al. 2007; Deckert 2009). Thus, this interaction will be dependent on blood circulation and retention time until the drug carrier reaches very close to the target area. Normally, negatively charged tumor cells require cationic particles for active targeting. However, intracellular penetration favors neutral particles (Campbell et al. 2002; Schmitt-Sody et al. 2003; Stylianopoulos et al. 2010). Regardless of these complications, modification of NLCs through coating or conjugation has been accomplished for targeted delivery to the site of interest (Aguilar 2012). Several different classes of modifiers are used for active targeting.

The hydrophobicity of the NLC surface attracts the mononuclear phagocyte system (MPS) to rapidly clear NLCs through opsonization (Grislain et al. 1983). To solve this problem, NLCs are coated with hydrophilic polymers

Table 3 Cryoprotectants used to freeze-dry different NLCs

Solid lipid	Liquid lipid	Drug	Cryoprotectant	Reference
Cholesterol	Oleic acid	Paclitaxel	25% sorbitol (w/w)	(Emami et al. 2012)
Cetyl palmitate	Octyldodecanol	Valproic acid	1% Avicel RC591 (w/v)	(Varshosaz, Eskandari, and Tabbakhian 2012)
Glycerol monostearate	Miglyol® 812 N	Isoliquiritigenin	5% lactose and 5% glucose (w/v)	(Zhang et al. 2013b)
Precirol® ATO 5	Miglyol® 812 N	Spironolactone	5/10/15% trehalose (w/v)	(Beloqui et al. 2014)
Precirol® ATO5	Capryol™ 90	Montelukast	3% mannitol (w/v)	(Patil-Gadhe et al. 2014)
Precirol® ATO 5	Miglyol® 812 N	LL37	15% trehalose (w/w)	(Garcia-Orue et al. 2016)
Lauric acid	Capryol™ 90	Rosuvastatin	5% mannitol (w/w)	(Patil-Gadhe and Pokharkar 2016)
Compritol® 888 ATO	Sesame oil	Tilmicosin	5% mannitol (w/v)	(Al-Qushawi et al. 2016)
Compritol® 888 ATO	Capmul® MCMC8	Tacrolimus	1% mannitol (w/v)	(Khan et al. 2016)

or surfactants, such as polyethylene glycol (PEG) (Bhadra et al. 2002; Mussi et al. 2015; Sun et al. 2018). Besides, carbohydrate such as chitosan, a cationic polysaccharide, is capable of interacting with anionic mucous layer, thus giving it a bio-adhesive property on epithelial cells (Casettari and Illum 2014; Chirio et al. 2014; Gartziandia et al. 2016; Singh et al. 2018). Mannose is another type of sugar used for the coating of NLCs due to overexpression of mannose receptors on the surfaces of macrophages at sites of inflammations (Wileman, Lennartz, and Stahl 1986; Xiao et al. 2013). Therefore, mannosylated NLCs are used to target areas of inflammation (Vieira et al. 2017;

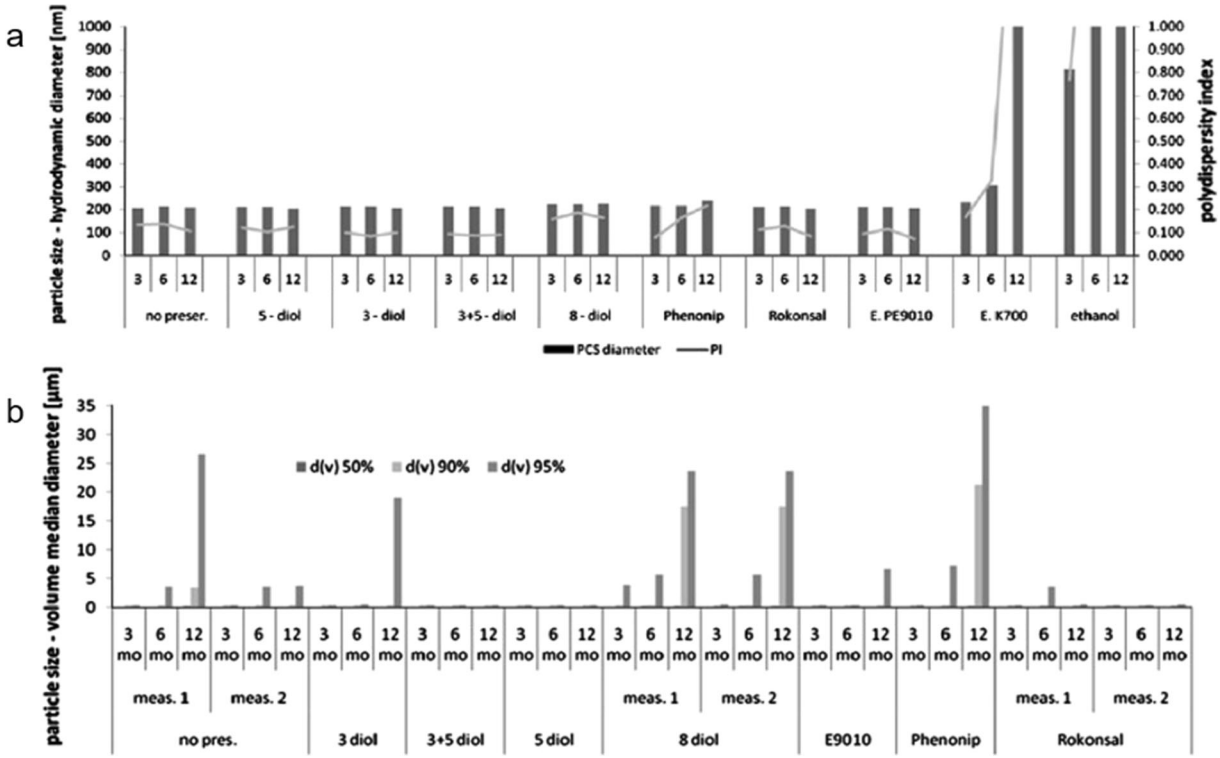


Fig. 9 The effects of different preservatives on the long-term stability of Q10-loaded NLC. **a** Hydrodynamic diameter and polydispersity indices of the particles measured via photon correlation spectroscopy (PCS). **b** Volume median diameter measured via laser diffractometry (LD) (Obeidat et al. 2010)

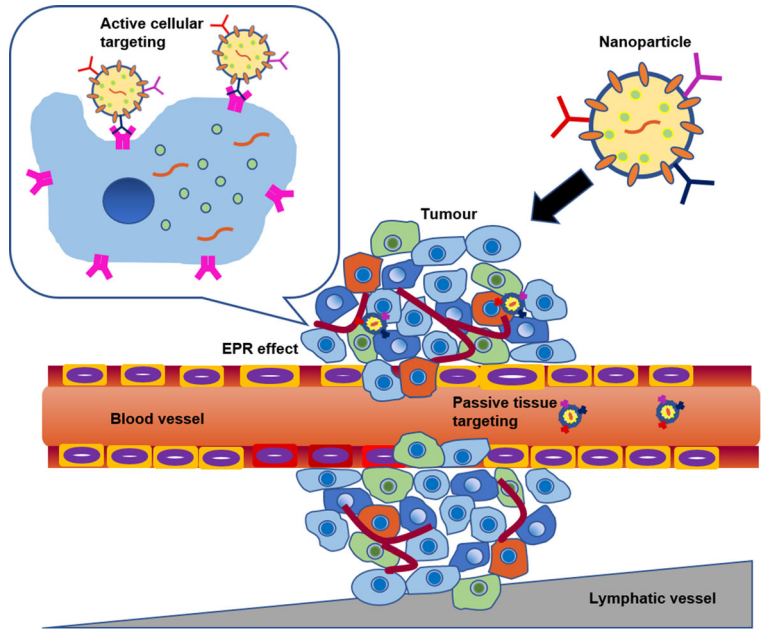


Fig. 10 Schematic illustration of active targeting vs passive targeting of nanoparticles. Nanoparticles can initiate two types of targeting which are active cellular targeting and passive tissue targeting. However, active targeting is preferred over passive targeting to avoid accumulation of nanoparticles in unintended areas

Sinhmar et al. 2018). Besides that, xyloglucan is a type of cellulose that acts as a hepatocyte-targeting carrier in hepatoma treatments (Liu et al. 2016).

As for protein-based modifiers, lectins bind easily to glycoproteins and glycolipids. These carbohydrate moieties are highly expressed in intestinal mucosa and lung tumors (Zhang et al. 2006; Pooja et al. 2016). Moreover, glycoproteins such as transferrin are used to target receptors on tumors and luminal membrane of brain endothelial cells (Fishman et al. 1987; Pardridge, Eisenberg, and Yang 1987; Maruyama et al. 2004; Gupta, Jain, and Jain 2007; Han et al. 2014). In some cases, cell-penetrating peptides are conjugated on the nanoparticle surface to target specific receptors on tumor cells (Huang et al. 2018). Several other ligands such as plerixafor, taurocholic acid, hyaluronic acid, and a combination of some of the above-mentioned ligands are used as modifiers to target specific receptors or antigens overexpressed on specific cells (Qu et al. 2015; Li et al. 2017; Tian et al. 2017; Wang et al. 2018). The different modifications of NLCs are detailed in Table 4.

Conclusions

Lipid-based nanoparticle systems for drug delivery have been studied extensively since the 1990s. SLNs as the first-generation lipid-based nanoparticles pioneered the way for a more efficient lipid carrier system, NLC. Over the years, NLCs with their augmented drug-loading capacity have shown promising results in the delivery of hydrophobic and hydrophilic drugs. NLC systems are produced to enhance drug dispersion in the aqueous environment, improve drug protection, prolong systemic circulation, target biological destination, and boost cellular drug uptake (Pathak and Thassu 2016). However, the variation in drug lipophilicity, lipid complexity, surfactant properties, and formulation methods have complicated the predictive steps of optimizing NLCs. This review has attempted to analyze and summarize the sequential steps during formulation to understand the trends and simplify the overall optimization process.

Beginning with materials selection, lipids and surfactants most compatible with the active compound are determined using simple visual or spectrophotometric solubility tests. Then, selection of a suitable formulation method between high-energy, low-energy, and organic solvent–based preparation is carried out. Once the materials and methods are finalized, RSM is used to optimize several independent variables that affect the dependent variables significantly. RSM can be further classified into statistical designs such as Plackett-Burman and 2^k factorial design for preliminary screening, or central composite and 3^k factorial designs for analyzing the relationship between factors and responses (Khuri and Mukhopadhyay 2010). Overall, statistical designs are used to minimize experimental runs and predict optimal formulations efficiently.

Table 4 Modifications of lipid nanoparticles to enhance active targeting for different biological purposes

Modifiers	Modification of NLCs	Results	Reference
Hydrophilic polymers			
Polyethylene glycol	Polyethylene glycol coating	Enhanced pharmacokinetics and tumor growth inhibition of doxorubicin-loaded NLCs	Mussi et al. 2015
	Polyargine and PEG-AEYLR (small peptide) coating	Improved in vivo tumor targeting ability compared to unmodified NLCs	Sun et al. 2018
Carbohydrates			
Chitosan	Chitosan coating	Glial cell–derived neurotrophic factor (GDNF)-NLCs showed behavioral improvement in a partially lesioned animal model of Parkinson's disease	Gartziandia et al. 2016
	Glycol chitosan coating	Increased retention of asenapine-loaded NLCs at the nasal epithelium	Singh et al. 2018
Mannose	Mannose coating	Induced decrease of intracellular growth of mycobacteria in tuberculosis treatment	Vieira et al. 2017
	Mannose coating	Enhanced accumulation of budesonide at inflamed colonic regions in rat models	Sinhmar et al. 2018
Cellulose	Xyloglucan coating	Modified 10-hydroxy-camptothecin (HCPT)-loaded NLCs exhibited superior cytotoxicity against drug-resistant HepG2 cells and higher in vivo therapeutic effect	Liu et al. 2016
Proteins			
Lectin	Wheat germ agglutinin-*N*--glutarylphosphatidyl--ethanolamine (WGA-N-glut-PE) conjugation	Improved oral absorption of insulin (SLN)	Zhang et al. 2006
	Wheat germ agglutinin (WGA) conjugation	Improved the oral bioavailability and lung targeting ability of paclitaxel	Pooja et al. 2016
Glycoprotein	Transferrin conjugation	Enhanced the brain uptake of quinine	Gupta, Jain, and Jain 2007
	Transferrin coating	Improved lung cancer cell-targeting of DNA-doxorubicin-loaded carriers	Han et al. 2014
Peptides	Cell-penetrating peptide RGERPPR	Increased accumulation of gambogic acid–loaded NLCs at tumor sites in the animal model	Huang et al. 2018
Other ligands/compounds			
	Hyaluronic acid	Improved anti-tumor activity of cisplatin/5-fluorouracil combination on gastric cancer cell lines	Qu et al. 2015
	Taurocholic acid (TCA) conjugation	Ligand-receptor interaction improved oral bioavailability of curcumin	Tian et al. 2017
	AMD3100 coating	Antagonized CXCR4 and prevented invasion of 4T1-luc cells in vitro and in vivo	Li et al. 2017
	Octaarginine, thiolytic cleavable polyethylene glycol (PEG) and targeting peptide	Improved accumulation and anti-tumor activity of paclitaxel-loaded NLCs at S180 tumor sites	Wang et al. 2018

The trends between independent variables and the nanoparticle characteristics should be comprehended, so that the ideal properties of NLCs are not compromised. The most studied particle characteristic, its size, is significantly affected by the types of lipids and surfactants, surfactant concentration, ratio of solid-to-liquid lipid, and drug concentration. Furthermore, polydispersity index that quantifies the aggregation tendency of the lipid particles is dependent on the types of surfactants and surfactant concentration. As for zeta potential which also predicts the stability of the system, the drug-to-lipid ratio, organic-to-aqueous phase ratio, sonication time, drug concentration, and types of lipids/surfactants are essential factors that affect it. The types of lipids/surfactants, the surfactant concentration, and the drug concentration are also found to modulate the entrapment efficiency of the carrier. In addition, the rate of drug release is altered by the types of lipids and the amounts of liquid lipid and surfactant.

Despite optimal achievements made in the nanoparticle's physicochemical properties, stability and targeted delivery of the system are essential requirements. Storage stability to prevent lipid aggregation and bacterial contamination is improved through the water removal process or the usage of preservatives. Water removal can be accomplished via spray drying or lyophilization using cryoprotectants. As for targeted delivery for various biomedical applications, modifications of NLCs via surface coating or conjugation are encouraged. Several surface modifiers made up of hydrophilic polymers, carbohydrates, proteins, and ligands have been studied for different modes of drug administration. Active targeting for diverse therapeutic purposes has seen improved outcomes when these modifiers are used with the NLC.

Conclusively, optimization of NLCs for different functions, treatments, and types of delivery is essential in the process of formulation. With or without the use of statistical methods, optimization can be accomplished by taking into consideration the characteristics necessary for effective drug delivery. Preliminary optimization through the selection of materials and methods, followed by modulation of key variables to avail optimized nanoparticle characteristics, and additional surface modifications for maximal targeted delivery are indispensable steps to achieve success in nanomedicine. The steps are extensive but with careful research and planning, optimization can be simplified without undermining the quality of the nanoparticle.

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Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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