Lipid Nanoparticle Preparations for Diverse Drug Administrations

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Abstract. The lipid nanoparticle (LNP) system serves as a crucial non-viral vector, playing a pivotal role in the realm of gene therapy delivery. Beyond its primary purpose, LNP modifications broaden its utility, spanning diverse application realms and administration methods. An array of LNP preparation technologies has emerged, tailored to meet varied requirements and categorized based on the specific mode of drug administration. This review systematically classifies common modes of administration suitable for LNPs, providing a comprehensive overview. Subsequently, it delves into specific examples of LNP preparation techniques designed for different administration routes, elucidating the achieved outcomes. The article meticulously analyzes the similarities and distinctions in LNP preparation methodologies across various routes of administration, with a keen focus on the unique demands posed by each route in terms of LNP composition and structure.

Keywords: LNP; Drug Delivery; Gene Therapy; Injectable Administrations; LNP Preparation.

1. Introduction

With the gradual drug development technology, gene therapy has emerged as a game changer in recent years[1]. The gene therapies are ranging from mRNA, siRNA and other nucleic acids expressed or regulated the expression of other genes in cells to achieve therapeutic effects[2]. However, the naked nucleic acids are fragile to nucleases. Safe, stable and efficient administration routes are therefore urgently needed to help the gene products to be taken up by the cells without being degraded.

The LNP vector is being well suited and aligning the above requirements. The lipid bilayer is formed by the self-assembly of various lipids, including phospholipids, cholesterol and other lipid components[3]. This bilayer structure provides stability to the nanoparticles and protects the therapeutic cargo from degradation, such as mRNA vaccines or other gene therapies[4]. Therapeutic nucleic acids, such as mRNA, are encapsulated within the aqueous core of the LNP. The lipid bilayer surrounding the core helps protect the mRNA from degradation and facilitates its administration to the target cell[5]. LNPs offer several advantages for therapeutic administration. They protect the encapsulated cargo from enzymatic degradation, enhance cellular uptake and internalization, and facilitate endosomal escape, which is essential for the therapeutic molecule to reach the cytoplasm of the target cell[6]. LNPs can be modified with different surface coatings or targeting ligands to enhance their specificity for a particular cell type or tissue. In the context of mRNA vaccines, LNPs have been used to encapsulate and deliver mRNA molecules encoding viral antigens, thereby triggering an immune response against the encoded antigen. LNPs can be administered by a variety of routes depending on the specific therapeutic application and target tissue, mainly including intravenous (IV) administration, subcutaneous (SC) administration, intramuscular (IM) administration, intraperitoneal (IP) administration, topical (TP) administration and inhalation (IH) administration[7]. Overall, lipid nanoparticles are versatile and efficient administration systems for therapeutic molecules, offering promising opportunities for advancing drug administration and personalized medicines.

This article summarized the above routes of administration and describes some specific examples of LNP Preparation. A discussion of the similarities and differences in the way LNP is synthesized by these routes of administration will help to provide a more comprehensive understanding of LNP Preparation. It will be useful for the selection of appropriate LNP Preparation and for the further development of LNP Preparation technology.

2. Versatile LNP Carriers for Different Injectable Administrations

2.1 Intravenous Administration

Intravenous injection of lipid nanoparticles involves injecting the nanoparticles directly into the bloodstream through a vein. Intravenous injection provides immediate and direct access to the systemic circulation of LNPs. Intravenous injection has high bioavailability, which means that a greater percentage of the administered dose reaches the target site than by other routes[8]. Intravenous LNPs avoid gastrointestinal degradation and hepatic enzymatic metabolism, thereby increasing the concentration of the drug in the blood[9].

2.2 Subcutaneous Administration

Subcutaneous delivery of lipid nanoparticles involves injecting the nanoparticles into the subcutaneous tissue layer or subcutaneously embedding a smart pump that releases the nanoparticles. The nanoparticles gradually release their payload to the surrounding tissue, thereby prolonging the therapeutic effect[10]. This is particularly useful for drugs or vaccines that need to be sustained in the body. Like intravenous administration, extracorporeal administration bypasses first-pass metabolism in the liver, resulting in higher bioavailability compared to oral administration. The relative sterility of subcutaneous tissue reduces the potential for introduction of pathogens during administration[11]. Subcutaneous injection does not require medical expertise and is suitable for patient self-injection. Subcutaneous injections allow for localized delivery of therapeutic agents and are therefore suitable for targeting specific tissues or sites of action. This is particularly relevant to dermatological conditions, as LNP acts directly on the affected area, thereby maximizing the concentration of the drug at the target site.

2.3 Intramuscular Administration

Intramuscular administration of lipid nanoparticles involves injecting the nanoparticles into the muscle. Intramuscular administration allows the LNP and its encapsulated payload to be rapidly absorbed into the bloodstream[12]. Muscle tissue has a rich blood supply, which helps the nanoparticles and therapeutic cargo to enter the systemic circulation quickly[13]. Intramuscular injections can accommodate larger administration volumes than subcutaneous injections. This is advantageous when administering larger doses or drugs that require larger doses to be administered effectively.

2.4 Intraperitoneal Administration

Intraperitoneal administration involves the injection of nanolipid particles into the abdominal cavity, which is the space surrounding the abdominal organs. IP administration results in a wide distribution of LNP throughout the abdominal cavity. The nanoparticles can encounter various organs and tissues within the abdominal cavity, allowing systemic administration to these sites[14]. The abdominal cavity provides direct access to specific organs in the abdomen, such as the liver, spleen, and intestines[14]. IP delivery can be targeted to these organs or to treat diseases affecting them. IP delivery can be used to locally treat diseases or conditions affecting abdominal organs[15]. IP delivery is commonly used in preclinical studies and animal experiments. It provides a convenient and effective way to assess the biodistribution, efficacy, and safety of LNP and its cargo in a variety of disease models[16]. It is important to note that IP administration is usually performed by trained healthcare professionals or researchers under controlled conditions. This route may not be suitable for human self-administration or routine clinical use.

2.5 Topical Administration

Topical administration of lipid nanoparticles involves the direct application of nanoparticles to the skin or mucous membranes. This route is used for topical administration of drugs to the site of application, e.g., creams or ointments for dermatological conditions[17]. LNPs can enhance the

penetration of therapeutic agents through the skin barrier[17]. The lipid component of the nanoparticles can interact with the lipids in the skin to facilitate the diffusion of the encapsulated drug or active ingredient into the lower layers of the skin[18]. Topical administration of LNPs is particularly relevant for the treatment of skin conditions such as eczema, psoriasis, acne or fungal infections[19]. LNPs can deliver anti-inflammatory agents, antibacterial drugs or other therapeutic compounds directly to the affected skin, improving local efficacy and minimizing systemic exposure[20]. Topical administration is usually simple and non-invasive, making it suitable for self-administration by patients. Creams, ointments, gels or sprays containing LNPs can be applied directly to the skin, enhancing patient compliance with treatment regimens. Topically administered LNPs provide an effective and convenient method of topical treatment, particularly for dermatological conditions. It allows for targeted administration, prolonged drug release and enhanced skin penetration, optimizing treatment outcomes while minimizing systemic exposure[20].

2.6 Inhalation Administration

Inhalation delivery of lipid nanoparticles refers to the delivery of nanoparticles into the respiratory tract via inhalation. Inhalation delivery provides targeted and direct delivery of LNP to the lungs and respiratory system[21]. The nanoparticles can reach the fine bronchioles and alveoli, which are the main sites of gas exchange in the lungs[22]. Therefore, inhalation drug delivery is particularly suitable for respiratory diseases or conditions that require localized treatment. The respiratory system provides a large surface area for drug absorption. The thin epithelium and highly dilated vasculature of the lungs allow LNP and its cargo to be rapidly absorbed into the bloodstream, thereby facilitating systemic distribution when needed[23]. Inhalation administration is non-invasive and does not require injections or surgery. It is well tolerated by patients and can be self-administered, thereby improving ease of administration and patient compliance. Inhaled LNP is particularly useful for the treatment of respiratory conditions such as asthma, chronic obstructive pulmonary disease or lung infections[24]. It is important to note that inhalation administration of LNP requires a specialized delivery system, such as a nebulizer or inhaler, to ensure proper particle size and deposition in the respiratory tract[24]. Inhalation administration should be performed under appropriate direction and supervision for optimal efficacy and safety.

3. Tailored Approaches for LNP Preparation Across Various Injection Route

Typically, the structure and composition of LNPs contain four major components: phospholipid bilayers, ionizable cationic lipids or lipid compounds, cholesterol, and others (such as polyethylene glycol (PEG)). And these components should be adjusted depending on the different drug molecules and the desired applications of LNPs.

3.1 Typical LNP Preparation Methods

There are many types of methods for LNP's preparation, each of them with its own advantages and limitations. Generally common methods of LNP preparation can be classified as: **a)** Thin-film hydration method: This method, widely used for lipid nanoparticle preparation[25], dissolves lipids and optional components (e.g., cholesterol, PEGylated lipids) in a volatile organic solvent[26]. This results in a thin lipid film on the surface of a container. The film is then hydrated with an aqueous solution, leading to the formation of multilamellar vesicles (MLVs)[27]. These are then converted to lipid nanoparticles through processes such as homogenization, sonication or extrusion; **b)** Microfluidic mixing: Microfluidics involves using small channels to accurately regulate fluid flow at the microscale[28]. Microfluidic mixing techniques enable the regulated mixing of lipid and aqueous phases that result in the production of small and uniform LNPs[29]. This method is advantageous as it can produce monodisperse nanoparticles and has the potential to be scaled up for commercial production[30]; **c)** Solvent dispersion method: In this method, lipids and the payload (e.g., drugs or genetic material) are dissolved in an organic solvent[31]. The organic solvent is then slowly mixed

with an aqueous solution, leading the lipids to form nanoparticles spontaneously as the solvent evaporates[32]. This method is relatively simple and can be employed to deliver different payloads[33]; **d)** Ethanol injection method: The method involves dissolving lipids in ethanol and injecting the resulting ethanol solution quickly into an aqueous phase under controlled conditions[27]. This rapid mixing technique results in the formation of LNPs, or lipid nanoparticles. It is recognised for its simplicity and high reproducibility[34]; **e)** Reverse phase evaporation method: In this method, an organic solvent containing dissolved lipids and payload is added dropwise to an excess of the aqueous phase[35]. This action creates a water-in-oil emulsion that is then reduced under pressure to create LNPs[36]. This technique suits the encapsulation of hydrophilic payloads; **f)** Emulsion method: This method entails creating a water-in-oil emulsion by blending an aqueous phase containing the payload with a lipid solution in an organic solvent[37]. Afterward, the emulsion undergoes homogenization or sonication to decrease droplet size and form LNPs. This method is advantageous for the encapsulation of both hydrophilic and hydrophobic payloads[38]; **g)** The freeze-drying technique: As for this technique, pre-formed LNPs are freeze-dried to get rid of water and produce a dry powder, which can enhance stability and improve the lifespan of LNPs[39]. Therefore, rendering them more suitable for storage and transportation. This approach can enhance the stability and life cycle of LNPs, thereby making them more fitting for storage and transportation[40].

The selected mode of administration may result in biased LNP synthesis, depending on the chosen synthetic method. The use of diverse and differentiated raw materials might contribute to enhancing LNP quality. Next, we will describe each of the six modes of administration mentioned in the previous paragraph.

3.2 Preparation of IV Administrated LNPs

Ranit Kedmi's group used high-purity hydrogenated soy phosphatidylcholine (HSPC) and 1,2-distearoylphosphatidylglycerol (DSPG) plus cholesterol (Chol) and 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP) to make the LNPs, an early ingredient used for intravenous administration[41]. Antibodies to polyethylene glycol may develop with repeated systemic administration[42]. Some LNPs suffer from aggregation in the liver, kidney and some cytotoxicity. Jong-Suep Baek *et al.* used 2-hydroxypropyl-β-cyclodextrin (HPCD)-modified paclitaxel (PTX) solid lipid nanoparticles (SLNs) to enhance PTX uptake and to reduce the concomitant nephrotoxicity associated with intravenous administration. The main components they chose were PTX, stearic acid, poloxamer, lecithin and HPCD[43]. Also, PEG may lead to hypersensitivity reactions, also known as complement activation-associated pseudo-allergy (CARPA) in specific individuals[44]. These findings have led to a few polymers based on amino acid derivatives that have been explored as alternatives to PEG. Polyinosine (pSar) is a polymer made from repeating units of the endogenous amino acid inosine (N-methylated glycine). An LNP for IV injection can be prepared from this substance with the cationic ionizable lipid DODMA, the co-lipid DSPC and the respective molar fraction 40/10/(50-x) of cholesterol, where x is the fraction of pSar lipid[45]. Most of the LNP preparation methods, used for this mode of administration, are thin-film hydration, ethanol injection and reverse phase evaporation methods[41, 43, 46].

3.3 Preparation of SC Administrated LNPs

Conventional LNPs formulations are poorly retained in lymph nodes, so SC delivery modalities were developed to enable LNPs to be transported to local lymph as well as distal tissues. Sam Chen *et al.* used DMAP-BLP, DSPC, cholesterol and PEG-DMG dissolved in ethanol at a molar ratio of 50: 10: (39.75 -x): (0.25 +x), respectively, where the amounts of cholesterol and PEG-DMG were varied accordingly ± x. This formulation was used as a basis for the synthesis of siRNA-bound LNP[47]. Because of the negative effect of PEGylated LNPs on the accelerated clearance of LNPs from the circulation after multiple lymphatic injections, as noted in one study[48]. It is recommended that formulations of SC administration LNPs be adjusted for PEG content. Considering that the tolerability of LNPs formulations is more important for SC administration, Nigel Davies *et al.*

mentioned that the effect could be enhanced by adding anti-inflammatory steroids to the formulation. They mentioned the use of roflumilone and budesonide. Lipophilicity was enhanced and the water solubility of the steroid reduced by adapting fatty acid ester prodrugs with different alkyl chain lengths[49]. This approach helped to improve their binding in LNP. And for synthetic formulations and methods that require binding of LNPs by siRNA, one can refer to the methods of Shubaash Anthiya's group[50]. Preparation was carried out using magnetic stirring. There are detailed steps and materials in their paper[50]. Microfluidic mixing is often used to prepare the LNPs used in this mode of delivery[10, 47, 51].

3.4 Preparation of IM Administrated LNPs

In IM-injected LNPs, Shuyu Xie *et al*. noted that fatty acids with different carbon chain lengths may affect release. They concluded that tetradecanoic acid had the highest release rate by experimenting with tetradecanoic acid, palmitic acid and stearic acid with carbon lengths of 14, 16 and 18, respectively[52]. The remaining components of their LNP were enrofloxacin and polyvinyl alcohol[52]. Intramuscular injections of anticancer drugs usually induce more serious side effects due to direct contact with the tissue and the initial burst effect. The novel double-reversible thermowell system (DRTG) technique developed by Fakhar ud Din *et al*. having higher anti-tumour efficacy without initial burst effects and toxicity [53]. In this technique thermos reversible LNP is used for drug delivery. They used tricaprin and triethanolamine as lipid mixture and Tween 80 and Span 80 need to be added[53]. Similarly, another study demonstrated the effect of Tween 20 and 80 as surfactants on the expression of LNPs injected at different sites[54]. They used LNPs with PEGylation properties to obtain the following results. tween 20 to improve the delivery of targeted genes from LNPs to lymph nodes after intramuscular injections. tween 80 could be used to form stable LNPs for spleen targeting, but with relatively low transfection efficiency[54]. A larger innovation was made by Huanzhen Ni *et al*. who designed a piperazine-derived lipid nanoparticles (Pi-LNPs)[55]. This LNP has an ionisable lipid consisting of a piperazine core and two tertiary amines as ionisable head groups attached to a hydrophobic carbon chain. The other parts are similar to normal LNPs, which are two PEG and two cholesterol variants[55]. Microfluidic mixing, reverse phase evaporation method and emulsion method have been employed to synthesise LNPs suitable for this mode of delivery[12, 15, 46, 56].

3.5 Preparation of IP Administrated LNPs

Stearic acid as the oil phase, Epicuron 200 as the surfactant, propionic acid, butyric acid and sodium taurocholate as the co-surfactants. This composition is used to deliver Baclofen by intraperitoneal injection[57]. This route is used when the oral dose is insufficient, and the drug action requires intrathecal access. R. Dal Magro *et al*. studied drug delivery to the brain through the blood-brain barrier by intraperitoneal injection of ApoE-modified LNPs. They used ApoE-derived peptides (SLN-mApoE) covalently surface-modified as a way to target the brain[58]. The main components of their LNP are dynasan 116, epikuron 200, and short-chain alcohols, but also require thiol-maleimide and phosphatidylcholine treatment[58]. It is the more complex LNP preparation process. The LNPs used in this mode of administration are typically synthesised by a microfluidic mixing, solvent dispersion method[59, 60].

3.6 Preparation of TP Administrated LNPs

Topical medication allows LNPs to carry drug molecules into the body through the skin or mucous membranes. Therefore, the particles of LNPs in this mode of drug delivery are generally small. Topical ocular drug delivery can likewise employ LNPs as novel drug carriers. In the summary by Jesus Alvarez-Trabado *et al*. they summaries the development of three generations of LNs and the preparation of many different emulsions[61]. Yingchen Chen *et al*. designed pharmaceuticals for the treatment of fungal skin infections manufactured by microemulsion technology using glycerol monostearate (GMS), glycerol esters and glycerol palmitostearate as solid lipid phases, Tween and

Cremophor series as surfactants and propylene glycol as a co-surfactant[62]. It can solve the problem of complexity of frequent medication, and at the same time, direct contact with the infected skin site increases the effect of the drug molecules. Another group used hydrophilic formulations of hydrogels to design LNPs. they dispersed melted palmitate (150 mg) in 7 ml of preheated water containing 47 mg of polysorbate 80 in a water bath at 10 above the melting point of the curd. Methotrexate (10% (w/w)) was incorporated when added to the lipid phase[63]. In another literature it is stated that nanostructured lipid carrier (NLC) lipid matrix is composed of biocompatible and biodegradable lipids with excellent tolerance and mixing ratios ranging from 70:30 to 99.9:0.1 solid lipid to oil. This colloidal system allows highly concentrated dispersion of lipid nanoparticles[64]. Freeze-drying method and microfluidic mixing has been used to synthesize LNPs employing TP delivery[65, 66].

3.7 Preparation of IH Administrated LNPs

Table 1. Correlations between administrations and applicable LNP preparation methods.

Administrations	Components	Preparation Methods
IV administration	HSPC and Chol at 4:1, HSPC: Chol: DSPG at 3:1:1, HSPC: Chol: DOTAP at 3:1:1[41]	Thin-Film Hydration Method[41]
	HEPC: CHOL: mPEG2000-DSPE at 1.85:1:0.15[72]	Thin-Film Hydration Method[72]
	Lipid: HSPC: Chol: PEG-lipid at 50:10:38.5:1.5[73]	Ethanol Injection Method[73]
	DLin-MC3-DMA: DSPC: Chol: DMG-PEG2k at 55:10:32.5:2.5[74]	Microfluidic Mixing[74]
	DLin-MC3-DMA: Chol: DSPC: DMPE-PEG 2000 at 50:38.5:10:1.5[75]	Microfluidic Mixing[75]
SC administration	DMAP-BLP: DSPC: Chol and PEG-DMG at 50:10:(39.75 - x): (0.25 + x), ± x refers to the quantity of Chol and PEG-DMG[76]	Microfluidic Mixing[76]
	Chol: DOPC: DMG-PEG2000: SS-OP at 20:27.5:1.5:52.5[77]	Microfluidic Mixing[77]
IM administration	lipid: DSPC: Chol: PEG-lipid at 50:10:38.5:1.5[78]	Microfluidic Mixing[78]
	Chol: DOPC: DMG-PEG2000: MC3 at 20:28.5:1.5:50[77]	Microfluidic Mixing[77]
	ionizable lipid: DSPC: Chol: PEG-lipid: GLA at 50:9.83:38.5:1.5:0.17[79]	Reverse Phase Evaporation Method[79]
IP administration	Lipid: DOPE: Chol: C14-PEG2000 at 35:16:46.5:2.5[80]	Solvent Dispersion Method[80]
	SS-cleavable and pH-activated lipid like material: DOPE: Chol: DMG-PEG2000 at 3:4:3:0.3[81]	Solvent Dispersion Method[81]
TP administration	Soybean phospholipids: α-tocopherol: Sodium cholate: Tween-80 at 3:0.1:1:3[82]	Microfluidic Mixing[82]
	Tripalmitin: SDC: Tween-20 at 10:6:5[83]	Freeze-drying Method[83]
IH administration	Lipid: structural lipid: Chol: PEG-DMG at 50:10:38.5:1.5[84]	Emulsion Method[84]
	Contains glyceryl monostearate: soybean phosphatidylcholine at 1:1[85]	Emulsion Method[85]

A Tam *et al*. RNA therapeutics transported through the respiratory tract could provide an early treatment strategy for viral respiratory infections such as neolocal pneumonia[67]. DSPC, DOPC, DOPE, DOPG, ESM, and DOPS, which may play an important role in determining uptake efficiencies in host cells[67]. An LNP can be obtained by double emulsion technique by adding 0.25 ml of TP5 solution (40 mg/ml) containing 20 mg of sodium cholate to 2.5 ml of chloroform and ethyl ether solution (1:1, v/v) containing 200 mg of monostearyl glycerol ester and soybean phosphatidylcholine (1:1, w/w, oil phase)[68]. The organic constituents evaporated to dry particles

can be used for pulmonary inhalation. A variety of methods for the preparation of aerosolized LNPs and lung transport are then summarized in the review by Neda Naseri *et al*[69]. Diana P. Gaspar *et al*. obtained the final SLN dispersion by using diphenyl dibenzoate or glycerol triturate as lipid component and Tween 80 as surfactant, allowing the hot nano emulsion to be cooled in an ice bath and gently stirred for 5 min[70]. They likewise tried empty glycerol dibehenate and empty glycerol tristate[70]. Emulsion method, solvent dispersion method and reverse phase evaporation method can be used to synthesize the LNPs used in this drug delivery method[68-71].Based on the preceding descriptions, it becomes evident that each administration typically had multiple approaches for preparing a LNP response. As a result, we have distilled the pertinent preparation methods for each administration and presented them in a comprehensive manner in table 1.

4. Conclusion

Although most of the LNPs components are in the common four categories, the best results can still be achieved by adjusting the composition when used in different situations. The use of different types of drug delivery methods can have the effect of facilitating drug transport, making LNPs more specific to a site, or controlling the duration of drug expression. Current research on the composition of various types of LNPs preparations has focused on adjusting the cationic lipid or PEG fraction used. Similarly, newer batches of LNPs may be more geared towards the impact of the transported siRNA with the shell produced. It serves as a guide in the absence of external modifications. Other studies have focused on making LNPs more targeted by attaching specific antibodies to the outside of liposomes.

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