

STABILIZATION OF LIPID NANOPARTICLE FORMULATIONS

RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of the filing date of U.S.

- 5 Provisional Application Serial Number 63/611,075, entitled “STABILIZATION OF LIPID NANOPARTICLE FORMULATIONS,” filed December 15, 2023, the entire contents of which are incorporated by reference herein.

BACKGROUND

- 10 Lipid nanoparticle (LNP) formulations are useful drug delivery systems for encapsulating various active pharmaceutical ingredients (APIs), for example, RNAs. LNP formulations commonly include aqueous buffer solutions, for example, phosphate buffer. However, current LNP formulations are susceptible to degradation and aggregate formation during storage at room temperature, thus improvements are needed.

SUMMARY

- 15 Aspects of the disclosure relate to compositions and methods for improving the stability of lipid nanoparticles (LNPs) and one or more active pharmaceutical ingredients (API) encapsulated therein. The disclosure is based, in part, on compositions that directly or indirectly reduce the degradation (e.g., oxidation, hydrolysis, etc.) of one or more lipids components of the lipid nanoparticle. In some embodiments, the compositions comprise a citrate,
- 20 ethylenediaminetetraacetic acid (EDTA), methionine, and/or tryptophan (e.g., a composition comprising a citrate drug product matrix, an EDTA drug product matrix, a methionine drug product matrix, and/or a tryptophan drug product matrix). In some embodiments, the citrate, EDTA, methionine, and/or tryptophan drug product matrix inhibits oxidation and/or hydrolysis of one or more lipids of the LNP and/or improves the stability of an API encapsulated within the
- 25 LNP. The disclosure also provides methods for storing compositions contemplated herein.

Accordingly, in some aspects, the disclosure provides a pharmaceutical composition comprising a lipid nanoparticle (LNP) comprising one or more ionizable lipids; and a drug product matrix, wherein the drug product matrix comprises: a concentration of citrate ranging from 5mM to 25 mM and a pH ranging from about 6.0 to about 8.5; a concentration of

- 2 -

ethylenediaminetetraacetic acid (EDTA) ranging from 10 μ M to 250 μ M and a pH ranging from about 6.0 to about 8.5; a concentration of methionine ranging from 5mM to 25 mM and a pH ranging from about 6.0 to about 8.5; or a concentration of tryptophan ranging from 5mM to 25 mM and a pH ranging from about 6.0 to about 8.5.

5 In some embodiments, the concentration of citrate, methionine, or tryptophan ranges between 5 mM and 20 mM. In some embodiments, the concentration of EDTA ranges between 25 μ M and 100 μ M.

 In some embodiments, the concentration of citrate methionine, or tryptophan is at least 5 mM. In some embodiments, the concentration of EDTA is at least 25 μ M.

10 In some embodiments, the concentration of citrate, methionine, or tryptophan is 10 mM. In some embodiments, the concentration of EDTA is 50 μ M.

 In some embodiments, the pH of the drug product matrix is about pH 6.9 to 7.5. In some embodiments, the pH of the drug product matrix is about pH 7.0 to 7.4. In some embodiments, the pH of the drug product matrix is about 7.0. In some embodiments, the pH of the drug
15 product matrix is about 7.4.

 In some embodiments, the drug product matrix further comprises one or more salts. In some embodiments, one or more salts comprises a sodium salt. In some embodiments, the sodium salt is NaCl.

 In some embodiments, the drug product matrix of further comprises phosphate-buffered
20 saline (PBS).

 In some embodiments, the one or more ionizable lipids comprises DLin-MC3-DMA (MC3) and/or Dioleoyl-3-trimethylammonium propane (DOTAP). In some embodiments, the one or more ionizable lipid consists of MC3 or DOTAP.

 In some embodiments, the LNP comprises one or more nucleic acids. In some
25 embodiments, the one or more nucleic acids comprises RNA. In some embodiments, the RNA is mRNA, siRNA, dsRNA, or miRNA. In some embodiments, the RNA is siRNA.

 In some embodiments, the composition has not been refrigerated or frozen. In some embodiments, the composition is stored at a temperature above 4 $^{\circ}$ C. In some embodiments, the temperature above 4 $^{\circ}$ C ranges from about 5 $^{\circ}$ C to about 30 $^{\circ}$ C.

30 In some aspects, the disclosure provides a container containing a pharmaceutical composition as described herein. In some embodiments, the container is a cartridge, prefilled

- 3 -

syringe, or glass vial. In some embodiments, the container is a prefilled syringe or a glass vial. In some embodiments, the container is a polymer vial. In some embodiments, a vial is a glass vial.

In some aspects, the disclosure provides a method for improving chemical stability of a lipid nanoparticle (LNP) pharmaceutical composition, the method comprising obtaining a non-citrate-containing LNP pharmaceutical composition comprising a non-citrate drug product matrix, a non-ethylenediaminetetraacetic acid (EDTA)-containing LNP pharmaceutical composition comprising a non-EDTA drug product matrix, a non-methionine-containing pharmaceutical composition comprising a non-methionine drug product matrix, or a non-tryptophan-containing pharmaceutical composition comprising a non-tryptophan drug product matrix ; and performing a buffer exchange reaction to replace the non-citrate drug product matrix of (i)(a) with a citrate excipient solution having a pH between 6.0 and 8.5 to obtain a citrate-including LNP pharmaceutical composition, the non-EDTA drug product matrix of (i)(b) with an EDTA excipient solution having a pH between 6.0 and 8.5 to obtain an EDTA-including LNP pharmaceutical composition, the non-methionine drug product matrix of (i)(c) with a methionine excipient solution having a pH between 6.0 and 8.5 to obtain a methionine-including LNP pharmaceutical composition, or the non-tryptophan drug product matrix of (i)(d) with a tryptophan excipient solution having a pH between 6.0 and 8.5 to obtain a tryptophan-including LNP pharmaceutical composition.

In some embodiments, the LNP pharmaceutical composition of (ii)(a), (ii)(b), (ii)(c), or (ii)(d) comprises one or more ionizable lipids comprising DLin-MC3-DMA (MC3) and/or Dioleoyl-3-trimethylammonium propane (DOTAP).

In some embodiments, the LNP pharmaceutical composition comprises one or more nucleic acids. In some embodiments, the one or more nucleic acids comprises RNA. In some embodiments, the RNA is mRNA, siRNA, dsRNA, or miRNA. In some embodiments, the RNA is siRNA.

In some embodiments, the citrate excipient solution, the EDTA excipient solution, the methionine excipient solution, or the tryptophan excipient solution has a pH of 6.9 to 7.5.

In some embodiments, the citrate excipient solution has a pH of 7.0, the EDTA excipient solution has a pH of 7.4, the methionine excipient solution has a pH of 7.4, or the tryptophan excipient solution of has a pH of 7.4.

- 4 -

In some embodiments, the LNP pharmaceutical composition has a concentration of citrate ranging from about 5 mM to about 25 mM, the LNP pharmaceutical composition has a concentration of EDTA ranging from about 10 μ M to about 250 μ M, the LNP pharmaceutical composition has a concentration of methionine ranging from about 5mM to about 25 mM, or the
5 LNP pharmaceutical composition has a concentration of tryptophan ranging from about 5 mM to about 25 mM.

In some embodiments, the LNP pharmaceutical composition has a concentration of citrate ranging from about 5 mM to 20 mM, a concentration of EDTA ranging from about 25 μ M and 100 μ M, a concentration of methionine ranging from about 5 mM to 20 mM, or a
10 concentration of tryptophan ranging from about 5 mM to 20 mM.

In some embodiments, the concentration of citrate in the LNP pharmaceutical composition is at least 5 mM, the concentration of EDTA is at least 25 μ M, the concentration of methionine is at least 10 mM, or the concentration of tryptophan is at least 5 mM.

In some embodiments, the concentration of citrate is 10 mM, the concentration of EDTA
15 is 50 μ M, the concentration of methionine is 10 mM, or the concentration of tryptophan is 10 mM.

In some embodiments, the buffer exchange reaction comprises contacting the non-citrate-containing LNP pharmaceutical composition, the non-EDTA-containing LNP pharmaceutical composition, the non-methionine-containing LNP pharmaceutical composition,
20 or the non-tryptophan-containing LNP pharmaceutical composition to a de-salting column.

In some embodiments, the buffer exchange reaction comprises contacting the non-citrate-containing LNP pharmaceutical composition, the non-EDTA-containing LNP pharmaceutical composition, the non-methionine-containing LNP pharmaceutical composition, or the non-tryptophan-containing LNP pharmaceutical composition to a dialysis tube.

25 In some embodiments, the buffer exchange reaction comprises collecting the LNP pharmaceutical composition in a container. In some embodiments, the container is a cartridge, prefilled syringe, or glass vial. In some embodiments, the container is a prefilled syringe or a glass vial. In some embodiments, the container is a polymer vial. In some embodiments, a vial is a glass vial.

- 5 -

In some embodiments, the method further comprises storing the LNP pharmaceutical composition at a temperature above 4 °C. In some embodiments, the temperature above 4 °C ranges from about 5 °C to about 30 °C.

In some embodiments, the obtained LNP pharmaceutical composition comprises fewer
5 hydrolyzed lipids relative to a LNP pharmaceutical composition stored in a phosphate buffer that does not contain citrate, EDTA, methionine, or tryptophan.

In some embodiments, the obtained LNP pharmaceutical composition comprises fewer oxidized lipids relative to a LNP pharmaceutical composition stored in a phosphate buffer that does not contain citrate, EDTA, methionine, or tryptophan.

10 In some embodiments, the obtained LNP pharmaceutical composition comprises LNPs having increased colloidal stability relative to LNPs stored in a pharmaceutical composition comprising phosphate buffer that does not contain citrate, EDTA, methionine, or tryptophan.

In some aspects, the disclosure provides a method for preparing a lipid nanoparticle (LNP) pharmaceutical composition, the method comprising preparing a first lipid composition
15 comprising one or more lipids; preparing a second lipid composition comprising a siRNA and an ionizable lipid; and mixing the first lipid composition and the second lipid composition, wherein the first lipid composition and second lipid composition are prepared using a citrate excipient solution having a pH between 6.0 and 8.5 and a citrate concentration of between 5mM and 25 mM, an ethylenediaminetetraacetic acid (EDTA) excipient solution having a pH between 6.0
20 and 8.5 and an EDTA concentration of between 10 µM and 250 µM, a methionine excipient solution having a pH between 6.0 and 8.5 and a methionine concentration of between 5mM and 25 mM, or a tryptophan excipient solution having a pH between 6.0 and 8.5 and a tryptophan concentration of between 5mM and 25 mM.

In some aspects, the disclosure provides a method for preparing a lipid nanoparticle
25 (LNP) pharmaceutical composition, the method comprising obtaining a lipid composition comprising a siRNA and an ionizable lipid, and mixing the lipid composition with a drug product matrix selected from: a citrate excipient solution having a concentration ranging from 5mM to 25 mM citrate and a pH ranging from about 6.0 to about 8.5; an ethylenediaminetetraacetic acid (EDTA) excipient solution having a concentration ranging from
30 10 µM to 250 µM EDTA and a pH ranging from about 6.0 to about 8.5; a methionine excipient solution having a concentration ranging from 5mM to 25 mM methionine and a pH ranging from

- 6 -

about 6.0 to about 8.5; or a tryptophan excipient solution having a concentration ranging from 5mM to 25 mM tryptophan and a pH ranging from about 6.0 to about 8.5.

BRIEF DESCRIPTION OF DRAWINGS

5 FIG. 1 shows representative structural features of two ionizable lipids, MC3 and DOTAP, used to manufacture lipid nanoparticles (LNPs).

 FIGs. 2A and 2B show the percentage of DOTAP (FIG. 2A) and MC3 (FIG. 2B) lipids, respectively, that are intact over a period of four weeks following storage in either glass (BT5933) or cyclic olefin polymer (COP) vials at different temperatures.

10 FIGs. 3A-3C show representative data indicating percentage hydrolysis (FIG. 3A) and percentage oxidation (FIG. 3B) of DOTAP lipids, and percentage oxidation (FIG. 3C) of MC3 lipids over a period of four weeks following storage in either glass (BT5933) or cyclic olefin polymer (COP) vials at different temperatures.

 FIG. 4 shows representative data from visual analyses of clarity of RNA-lipid
15 nanoparticle (LNP) compositions stored for four weeks at RT in control buffer (phosphate-buffered saline (PBS)) or a buffer comprising an excipient, such as ethylenediaminetetraacetic acid (EDTA), methionine, or tryptophan.

 FIG. 5 shows representative data from analyses of RNA-LNP size and polydispersity following storage for four weeks at RT in compositions comprising control buffer (PBS) or a
20 buffer comprising an excipient, such as ethylenediaminetetraacetic acid (EDTA), methionine, or tryptophan.

 FIG. 6 shows representative data from analyses of encapsulation efficiency and RNA concentration ([RNA]) following storage for four weeks at RT in compositions comprising control buffer (PBS) or a buffer comprising an excipient, such as ethylenediaminetetraacetic
25 acid (EDTA), methionine, or tryptophan.

 FIG. 7 shows representative data from analyses of the production of dienone degradation byproducts in RNA-LNP compositions comprising MC3 and stored at RT for four weeks in control buffer (PBS) or a buffer comprising an excipient, such as ethylenediaminetetraacetic acid (EDTA), methionine, or tryptophan.

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- 7 -

DETAILED DESCRIPTION

Aspects of the disclosure relate to compositions and methods for improving the stability of lipid nanoparticles and one or more active pharmaceutical ingredients (API) encapsulated therein. The disclosure is based, in part, on compositions comprising components (e.g.,
5 excipients such as citrate, ethylenediaminetetraacetic acid (EDTA), methionine, and/or tryptophan in solutions at certain pH ranges, etc.) that directly or indirectly reduce the degradation (e.g., oxidation, hydrolysis, etc.) of one or more lipids components of the LNP. The disclosure also provides methods for storing compositions contemplated herein as well as methods for improving the stability of the API.

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Lipid Nanoparticles

Aspects of the disclosure relate to compositions comprising lipid nanoparticles (LNPs). As used herein, the term “LNP” refers to any particle comprising at least one lipid component having an average mean diameter of less than 1000 nanometers. Exemplary LNPs include, but
15 are not limited to, micelles, liposomes, lipoplexes, and solid lipid nanoparticles, or derivatives thereof. LNPs may have any morphology and structure known in the art. For example, in some embodiments, LNPs are nanospheres, nanorods, nanochains, nanostars, nanoflowers, nanoreefs, nanowhiskers, nanofibers, and nanoboxes. In some embodiments, other morphologies and structures are also possible.

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In some embodiments, an LNP comprises a micelle. A micelle may be a micelle or a reverse-micelle. In some embodiments, micelles are aggregates of amphipathic lipids dispersed in a liquid forming a colloidal suspension. In some embodiments, amphipathic lipids comprise a hydrophilic head group and a hydrophobic tail group that, when dispersed in a water, spontaneously assembles into structures that expose the head groups to the water phase and bury
25 the hydrophobic tails group into the core of the structure. In some embodiments, the lipids are dispersed into an oil phase and spontaneously assemble into structures that expose the hydrophobic tail groups and bury the hydrophilic head groups (e.g., the lipids form a reverse micelle). In some embodiments, combinations of structures are also possible. For example, in some embodiments, lipids may be added to emulsions, for example, a water-in-oil emulsion, an
30 oil-in-water emulsion, or the like. In some embodiments, lipids added to an oil-in-water emulsion (e.g., water is the continuous phase and oil is the dispersed phase) spontaneously

- 8 -

assemble at the water-oil interface with the hydrophilic heads pointed toward the water phase and the hydrophilic tails oriented toward the oil phase. In this configuration, the lipids form a monolayer around the oil droplet transforming it from a hydrophobic surface into a hydrophilic surface, thus stabilizing the oil droplets in the water phase (e.g., preventing the oil droplets from coalescing). In some embodiments, lipids added to water-in-oil emulsion (e.g., oil is the continuous phase and water is the dispersed phase) form a monolayer around the water droplets dispersed in an oil phase. In some embodiments, the lipid monolayer may comprise one or more targeting molecules (e.g., antibodies or fragments thereof, cell targeting peptides, etc.), drugs, or other agents (e.g., polyethylene glycol). Combinations of emulsions are also possible in some embodiments. For example, in some embodiments, water-in-oil-in-water emulsions are contemplated herein. Such a configuration would produce stabilized water droplets encapsulated within a larger oil droplet. Other combinations are also possible in some embodiments. For example, in some embodiments, oil-in-water-in-oil emulsions may be used to create the LNPs disclosed herein.

In some embodiments, the LNP comprises a liposome. In some embodiments, a liposome is an artificial vesicle having at least one lipid bilayer. In some embodiments, the liposome is a multilamellar vesicle (MLV). In some embodiments, the liposome is a large unilamellar vesicle (LUV). In some embodiments, the liposome is a small unilamellar vesicle (SUV). In some embodiments, MLVs are large “onion-like” structures comprising several lamellar phase lipid bilayers (e.g., mean diameters greater than 1000 nanometers). In some embodiments, LUVs comprise large unilamellar vesicles (e.g., average diameters of between 100 to 200 nanometers). In some embodiments, SUVs comprise small unilamellar vesicles (e.g., mean diameter of between 15 to 30 nanometers). In some embodiments, the lipid bilayer may comprise one or more targeting molecules (e.g., antibodies or fragments thereof, cell targeting peptides, etc.), drugs, or other agents (e.g., polyethylene glycol).

In some embodiments, the LNP comprises a lipoplex. As used herein, the term “lipoplex” refers to a complex formed between a charged liposome (e.g., a cationic or anionic lipid) and at least one oppositely charged component (e.g., a nucleic acid, such as a siRNA). As used herein, the at least one oppositely charged component may be a small molecule drug, a polynucleotide (e.g., DNA, RNA, siRNA, miRNA, etc.), a peptide, a polypeptide, a protein (e.g., an antibody), a polymer, or a polysaccharide comprising at least one cationic (e.g., -NH₃⁺)

- 9 -

and/or anionic (e.g., COO-) group. Without wishing to be bound by theory, it is believed that the complex is formed due to the electrostatic interaction between the opposing charge groups between the charged lipid in the liposome bilayer and the oppositely charged component.

In some embodiments, any one of the LNPs disclosed herein comprise solid lipid nanoparticles. In some embodiments, solid lipid nanoparticles comprise a solid lipid core matrix capable of solubilizing lipophilic molecules (e.g., oils, lipids, charged lipid-complexes, DNA, RNA, etc.). In some embodiments, the solid lipid core is stabilized by a lipid monolayer (e.g., similar to micelles). In some embodiments, the lipid monolayer may comprise one or more targeting molecules (e.g., antibodies or fragments thereof, cell targeting peptides, etc.), drugs, or other agents (e.g., polyethylene glycol).

In some embodiments, the LNPs of the present disclosure have an average mean diameter of greater than or equal to 1 nanometer, greater than or equal to 5 nanometers, greater than or equal to 10 nanometers, greater than or equal to 50 nanometers, greater than or equal to 100 nanometers, greater than or equal to 200 nanometers, greater than or equal to 300 nanometers, greater than or equal to 400 nanometers, greater than or equal to 500 nanometers, greater than or equal to 600 nanometers, greater than or equal to 700 nanometers, greater than or equal to 800 nanometers, greater than or equal to 900 nanometers, or greater than or equal to 1000 nanometers. In some embodiments, the LNPs have an average mean diameter of less than or equal to 1000 nanometers, less than or equal to 900 nanometers, less than or equal to 800 nanometers, less than or equal to 700 nanometers, less than or equal to 600 nanometers, less than or equal to 500 nanometers, less than or equal to 400 nanometers, less than or equal to 300 nanometers, less than or equal to 200 nanometers, less than or equal to 100 nanometers, less than or equal to 50 nanometers, less than or equal to 10 nanometers, less than or equal to 5 nanometers, or less than or equal to 1 nanometer.

In some embodiments, the LNPs have an average mean diameter of between 1 nanometer and 1000 nanometers, between 1 nanometer and 350 nanometers, between 1 nanometer and 300 nanometers, between 1 nanometer and 250 nanometers, between 1 nanometer and 200 nanometers, between 1 nanometer and 150 nanometers, between 1 nanometer and 100 nanometers, or between 1 nanometer and 50 nanometers. In some embodiments, the average mean particle diameter is between 100 nanometers and 900 nanometers, between 200 nanometers and 800 nanometers, between 300 nanometers and 700 nanometers, or between 400

- 10 -

nanometers and 600 nanometers. LNPs with other average mean diameters are also possible, in some embodiments.

In some embodiments, LNPs comprises one or more lipids (e.g., one or more different types of lipids, for example 1, 2, 3, 4, 5, or more different lipids). As used herein, the term lipid
5 refers to any class of organic compounds that are fatty acids or their derivatives and are insoluble in water but soluble in organic solvents (e.g., waxes, fats, oils, hormones, lipid membranes, etc.). In some embodiments, the lipids comprise amphiphilic lipids. In some
embodiments, the lipids comprise neutral lipids. In some embodiments, the lipids comprise one or more ionizable lipids. In some embodiments, the one or more ionizable lipids comprises
10 cationic lipids, anionic lipids, or a combination thereof. In some embodiments, the lipids comprise functionalized lipids. In some embodiments, functionalized lipids comprise one or more reactive groups (e.g., amines, carboxyl groups, etc.). In some embodiments, the lipids comprise lipid-conjugates, for example, lipid-polyethylene glycol. In some embodiments, the lipid-conjugates comprise more than one conjugate.

In some embodiments, the lipid is 1,2-dioleoyl-3-trimethylammonium-propane or a derivative thereof (herein "DOTAP"). In some embodiments, the lipid is 4-(dimethylamino)-
butanoic acid, (10Z,13Z)-1-(9Z,12Z)-9,12-octadecadien-1-yl-10,13-nonadecadien-1-yl ester or a derivative thereof (e.g., herein "Dlin-MC3-DMA" or "MC3"). In some embodiments, the lipid is
15 Heptadecan-9-yl 8-[(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino]octanoate or a derivative thereof (herein "SM-102"). In some embodiments, the lipid is [(4-Hydroxybutyl)azanediyl]di(hexane-6,1-diyl) bis(2-hexyldecanoate) or a derivative thereof
(herein "Alc-0315"). In some embodiments, the lipid is cholesterol or a derivative thereof. In some embodiments, the lipid is (1,2-distearoyl-sn-glycero-3-phosphocholine) or a derivative thereof (herein "DSPC"). In some embodiments, the lipid is 1,2-dimyristoyl-rac-glycero-3-
20 methoxypolyethylene glycol-2000 or a derivative thereof (herein "DMG-PEG-2000").

In some embodiments, the one or more ionizable lipids comprises MC3 or DOTAP.

In some embodiments, compositions of the disclosure comprise LNP compositions with varying ratios of the one or more lipids. Any suitable ratio known in the art may be used to
produce the LNPs disclosed herein. Those familiar with the art, will appreciate that the sum of
30 the ratios from each lipid component cannot exceed 100% (e.g., mole %, weight %, mass %, volume %, etc.). For example, in some embodiments, the percent contribution of each lipid in

the LNP composition is greater than or equal to 1%, greater than or equal to 5%, greater than or equal to 10%, greater than or equal to 15%, greater than or equal to 20%, greater than or equal to 25%, greater than or equal to 30%, greater than or equal to 35%, greater than or equal to 40%, greater than or equal to 45%, greater than or equal to 50%, greater than or equal to 55%, greater than or equal to 60%, greater than or equal to 65%, greater than or equal to 70%, greater than or equal to 75%, greater than or equal to 80%, greater than or equal to 85%, greater than or equal to 90%, greater than or equal to 95%, or greater than or equal to 100%. In some embodiments, the percent contribution (e.g., mole %) of each lipid in LNP composition is less than or equal to 100%, less than or equal to 95%, less than or equal to 90%, less than or equal to 85%, less than or equal to 80%, less than or equal to 75%, less than or equal to 70%, less than or equal to 65%, less than or equal to 60%, less than or equal to 55%, less than or equal to 50%, less than or equal to 45%, less than or equal to 40%, less than or equal to 35%, less than or equal to 30%, less than or equal to 25%, less than or equal to 20%, less than or equal to 15%, less than or equal to 10%, less than or equal to 5%, or less than or equal to 1%.

In some embodiments, the method comprises LNP compositions comprising one or more nucleic acids (e.g., siRNA, mRNA, dsRNA, or miRNA). In some embodiments, the concentration of the one or more nucleic acid in the LNP composition is between about 1 ng/mL and 50 mg/mL. In some embodiments, the concentration of the one or more nucleic acids in the LNP composition is between about 1 mg/mL and 50 mg/mL. In some embodiments, the concentration of the one or more nucleic acids in the LNP composition is between about 500 μ g/mL and about 20 mg/mL (e.g., about 500 μ g/mL, about 1 mg/mL, about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 11 mg/mL, about 12 mg/mL, about 13 mg/mL, about 14 mg/mL, about 15 mg/mL, about 16 mg/mL, about 17 mg/mL, about 18 mg/mL, about 19 mg/mL, or about 20 mg/mL). In some embodiments, the concentration of the one or more nucleic acids in the LNP composition is between about 500 μ g/mL and 3 mg/mL (e.g., about 500 μ g/mL, about 1 mg/mL, about 1.5 mg/mL, about 2 mg/mL, or about 3 mg/mL). In some embodiments, the concentration of the one or more nucleic acids in the LNP composition is between about 1 mg/mL and about 5 mg/mL, for example about 1 mg/mL, about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, or about 5 mg/mL. In some embodiments, the concentration of the one or more nucleic acids in the LNP composition is between about 1.5 mg/mL and 2.5

- 12 -

mg/mL (e.g., about 1.5 mg/mL, about 1.6 mg/mL, about 1.7 mg/mL, about 1.8 mg/mL, about 1.9 mg/mL, about 2.0 mg/mL, about 2.1 mg/mL, about 2.2 mg/mL, about 2.3 mg/mL, about 2.4 mg/mL, or about 2.5 mg/mL).

In some embodiments, an LNP comprises a stabilizer. Without wishing to be bound by theory, stabilizers are art recognized compounds capable of improving the stability of any one of the LNPs disclosed herein. In some embodiments, the stabilizer comprises a lipid-polyethylene glycol conjugate. In some embodiments, the stabilizer comprises sucrose. Other stabilizers are also possible in some embodiments.

In some embodiments, the LNPs disclosed herein further comprise an active pharmaceutical ingredient (API). In some embodiments, the LNPs disclosed herein further comprise one or more nucleic acids. In some embodiments, the API comprises one or more nucleic acids. In some embodiments, the one or more nucleic acids comprises RNA. In some embodiments, the RNA is a messenger ribonucleic acid (herein “mRNA”), a small interfering ribonucleic acid (herein “siRNA”), a double stranded ribonucleic acid (herein “dsRNA”), or a micro ribonucleic acid (herein “miRNA”).

In some embodiments, the nucleic acid is a siRNA. siRNAs are art recognized non-coding double-stranded RNAs that operate within the RNA interference pathway. Without being bound by theory, it is believed that they interfere with expression of specific genes with complementary nucleotide sequences by degrading mRNA after transcription, thus preventing translation. In some embodiments, the siRNA is a nucleic acid therapeutic that targets (e.g., silences or inhibits) a gene associated with a human disease or disorder.

In some embodiments, the one or more nucleic acids is a micro-RNA (herein “miRNA”). miRNAs are art recognized single stranded, non-coding RNA molecules involved in RNA silencing and post-transcriptional regulation of gene expression. Without being bound by theory, it is believed that miRNAs base-pair to complementary sequences in mRNAs which allows them to silence the mRNA molecule via cleavage of mRNA strand into two pieces or destabilization of mRNA by shortening its poly(A) tail.

In some embodiments, the one or more nucleic acids are encapsulated within any one of the LNPs disclosed herein. In some embodiments, the one or more nucleic acids may be encapsulated within any structure of any one of the LNPs disclosed herein. For example, in some embodiments, the one or more nucleic acids may be encapsulated within the hydrophobic

- 13 -

lipid bilayer of a liposome or a lipoplex. In some embodiments, the one or more nucleic acids may be encapsulated within the hydrophilic core of a liposome or lipoplex. In some embodiments, the one or more nucleic acids may be encapsulated within the lipophilic core of a solid lipid nanoparticle. In some embodiments, the one or more nucleic acids may be
5 encapsulated within one or more reverse micelles encapsulated within a micelle. In some embodiments, the one or more nucleic acids may be electrostatically bound to the outside surface of an LNP. Other configurations are also possible in some embodiments.

Drug Product Matrix

10 Aspects of the disclosure relate to compositions (e.g., LNPs) comprising certain excipients, such as citrate, ethylenediaminetetraacetic acid (EDTA), methionine, and/or tryptophan drug product matrix. In some embodiments, the excipients are present in a solution (e.g., an aqueous solution, for example an excipient-containing solution). In some embodiments, an excipient-containing solution comprises one or more excipients selected from citrate,
15 ethylenediaminetetraacetic acid (EDTA), methionine, and/or tryptophan, a diluent, such as water and one or more buffering agents, and, optionally, one or more active pharmaceutical ingredients (API) (e.g., a LNP containing an API). In some embodiments, such a composition is referred to as a drug product matrix.

As used herein, the term “citrate” refers to an organic compound comprising the salts
20 (e.g., sodium citrate, potassium citrate, calcium citrate, etc.), esters (e.g., tributyl citrate, acetyl tributyl citrate, and triethyl citrate, etc.), and/or a polyatomic anion of citric acid (e.g., a compound comprising the formula $C_3H_5O(COO)_3^{-3}$). As used herein, the term “ethylenediaminetetraacetic acid” or “EDTA” refers to an aminopolycarboxylic acid comprising the formula $[CH_2N(CH_2CO_2H)_2]_2$ and salts thereof (e.g., a disodium salt of EDTA or a calcium
25 disodium salt of EDTA). As used herein, the term “methionine” refers to the essential amino acid comprising an alpha-amino group, a carboxylic acid group, and a *S*-methyl thioether side chain comprising the formula C_2H_7S . In some embodiments, the methionine is L-methionine. In some embodiments, the methionine is D-methionine. In some embodiments, the methionine is a combination of L-methionine and D-methionine. As used herein, the term “tryptophan” refers to
30 the essential amino acid comprising an alpha-amino group, a carboxylic acid group, and an indole side chain. In some embodiments, the tryptophan is L-tryptophan. In some embodiments,

- 14 -

the tryptophan is D-tryptophan. In some embodiments, the tryptophan is a combination of L-tryptophan and D-tryptophan.

In some embodiments, an excipient-containing solution (e.g., drug product matrix) comprises a citrate, methionine, and/or tryptophan concentration of between 5 mM and 50 mM.

- 5 In some embodiments, a drug product matrix has a citrate, methionine, and/or tryptophan concentration of between 5 mM and 50 mM, between 10 mM and 40 mM, or between 20 mM and 30 mM. In some embodiments, the concentration of the citrate, methionine, and/or tryptophan in the drug product matrix is 10 mM. In some embodiments, the drug product matrix has an EDTA concentration of between 10 μ M and 250 μ M. In some embodiments, the drug product matrix has an EDTA concentration of between 20 μ M and 200 μ M, between 25 μ M and 150 μ M, between 30 μ M and 90 μ M, between 35 μ M and 75 μ M, between 40 μ M and 60 μ M, or between 45 μ M and 55 μ M. In some embodiments, the drug product matrix has a EDTA concentration of 50 μ M. Other combinations are also possible in some embodiments.

- 15 In some embodiments, a drug product matrix (e.g., citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, or tryptophan drug product matrix) has a pH value of between 6.0 to 8.5 (e.g., 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, or 8.5). In some embodiments, a drug product matrix (e.g., citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, or tryptophan drug product matrix) has a pH value of between 6.0 to 8.0 (e.g., 20 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0). In some embodiments, a drug product matrix (e.g., citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, or tryptophan drug product matrix) has a pH value of between 6.5 to 8.0 (e.g., 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0). In some embodiments, a drug product matrix (e.g., citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, or tryptophan drug product matrix) has a pH value of between 6.5 to 7.5 (e.g., 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, or 7.5). In some embodiments, a drug product matrix (e.g., citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, or tryptophan drug product matrix) has a pH value of between 7.0 to 8.0 (e.g., 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0). In 30 some embodiments, a drug product matrix (e.g., containing citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, or tryptophan drug product matrix) has a

- 15 -

pH value of between 7.0 to 7.5 (e.g., 7.0, 7.1, 7.2, 7.3, 7.4, or 7.5). In some embodiments, a drug product matrix (e.g., citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, or tryptophan drug product matrix) has a pH value of about 7.0 or about 7.4.

In some embodiments, a drug product matrix comprises one or more salts. In some
5 embodiments, the drug product matrix comprises sodium chloride. In some embodiments, the
drug product matrix comprises phosphate and one or more excipients. In some embodiments, the
concentration of the one or more salts is between 10 mM and 300 mM (e.g., about 10 mM, 20
mM, 40 mM, 80 mM, 100 mM, 120 mM, 140 mM, 160 mM, 180 mM, 200 mM, 220 mM, 240
mM, 260 mM, 280 mM, or 300 mM). Other concentrations are also possible in other ranges
10 and/or combinations in some embodiments.

In some embodiments, a drug product matrix is a citrate-containing drug product matrix
(e.g., a citrate drug product matrix). In some embodiments, a citrate drug product matrix has a
citrate concentration of between 5 mM and 50 mM. In some embodiments, the citrate
concentration is greater than or equal to 5 mM, greater than or equal to 10 mM, greater than or
15 equal to 20 mM, greater than or equal to 30 mM, greater than or equal to 40 mM, or greater than
or equal to 50 mM. In some embodiments, the citrate concentration is less than or equal to 50
mM, less than or equal to 40 mM, less than or equal to 30 mM, less than or equal to 20 mM, less
than or equal to 10 mM, or less than or equal to 5 mM. In some embodiments, the concentration
of the citrate in the citrate drug product matrix is 10 mM. Other combinations are also possible
20 in some embodiments.

In some embodiments, a citrate drug product matrix has a citrate concentration of
between 5 mM and 50 mM, between 10 mM and 40 mM, or between 20 mM and 30 mM. Other
ranges are also possible in some embodiments.

In some embodiments, a citrate drug product matrix has a citrate concentration of
25 between 5 mM and 25 mM, between 10 mM and 20 mM, between 12 mM and 18 mM, or
between 14 mM and 16 mM. In some embodiments, a citrate drug product matrix has a citrate
concentration of at least 5 mM, at least 10 mM, at least 20 mM, at least 25 mM, or at least 30
mM. In some embodiments, the citrate drug product matrix drug product matrix has a citrate
concentration of 10 mM.

30 In some embodiments, a citrate drug product matrix has a pH value of between 6.0 to 8.5
(e.g., 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0,

- 16 -

8.1, 8.2, 8.3, 8.4, or 8.5). In some embodiments, the pH is greater than or equal to 6.0, greater than or equal to 6.1, greater than or equal to 6.2, greater than or equal to 6.3, greater than or equal to 6.4, greater than or equal to 6.6, greater than or equal to 6.7, greater than or equal to 6.8, greater than or equal to 6.9, greater than or equal to 7.0, greater than or equal to 7.1, greater than
5 or equal to 7.2, greater than or equal to 7.3, greater than or equal to 7.4, greater than or equal to 7.5, greater than or equal to 7.6, greater than or equal to 7.7, greater than or equal to 7.8, greater than or equal to 7.9, greater than or equal to 8.0, greater than or equal to 8.1, greater than or equal to 8.2, greater than or equal to 8.3, greater than or equal to 8.4, or greater than or equal to 8.5. In some embodiments, the pH is less than or equal to 6.0, less than or equal to 6.1, less than
10 or equal to 6.2, less than or equal to 6.3, less than or equal to 6.4, less than or equal to 6.5, less than or equal to 6.6, less than or equal to 6.7, less than or equal to 6.8, less than or equal to 6.9, less than or equal to 7.0, less than or equal to 7.1, less than or equal to 7.2, less than or equal to 7.3, less than or equal to 7.4, less than or equal to 7.5, less than or equal to 7.6, less than or equal to 7.7, less than or equal to 7.8, less than or equal to 7.9, or less than or equal to 8.0. In some
15 embodiments, a citrate drug product matrix has a pH value of between 6.5 to 7.5 or 7.0 to 7.5. In some embodiments, a citrate drug product matrix has a pH of about 7.0. In some embodiments, the pH ranges from about pH 3.0 to about pH 7.0.

In some embodiments, a citrate drug product matrix comprises one or more salts. In some embodiments, the citrate drug product matrix comprises sodium chloride. In some
20 embodiments, the concentration of the one or more salts is between 10 mM and 300 mM. In some embodiments, the concentration of the one or more salts is greater than or equal to 10 mM, greater than or equal to 20 mM, greater than or equal to 40 mM, greater than or equal to 80 mM, greater than or equal to 100 mM, greater than or equal to 120 mM, greater than or equal to 140 mM, greater than or equal to 160 mM, greater than or equal to 180 mM, greater than or equal to
25 200 mM, greater than or equal to 220 mM, greater than or equal to 240 mM, greater than or equal to 260 mM, greater than or equal to 280 mM, or greater than or equal to 300 mM. In some embodiments, the concentration of the one or more salts in the citrate drug product matrix is less than or equal to 300 mM, less than or equal to 280 mM, less than or equal to 260 mM, less than or equal to 240 mM, less than or equal to 220 mM, less than or equal to 200 mM, less than or
30 equal to 180 mM, less than or equal to 160 mM, less than or equal to 140 mM, less than or equal to

- 17 -

to 120 mM, less than or equal to 100 mM, less than or equal to 80 mM, less than or equal to 40 mM, less than or equal to 20 mM, or less than or equal to 10 mM.

In some embodiments, the concentration of the one or more salts in a citrate drug product matrix is between 10 mM and 300 mM, between 20 mM and 280 mM, between 40 mM and 260 mM, between 80 mM and 240 mM, between 100 mM and 220 mM, between 120 mM and 200 mM, or between 140 mM and 180 mM. Other concentrations are also possible in other ranges and/or combinations in some embodiments.

In some embodiments, a drug product matrix comprising citrate (e.g., a citrate drug product matrix described herein) does not comprise at least one of: EDTA; methionine; and tryptophan. In some embodiments, a drug product matrix comprising citrate (e.g., a citrate drug product matrix described herein) does not comprise more than one of: EDTA; methionine; and tryptophan. In some embodiments, a drug product matrix comprising citrate (e.g., a citrate drug product matrix described herein) does not comprise EDTA, methionine, and tryptophan. In some embodiments, a drug product matrix comprising citrate (e.g., a citrate drug product matrix described herein) does not comprise histidine.

In some embodiments, a drug product matrix is an EDTA-containing drug product matrix (e.g., EDTA drug product matrix). In some embodiments, an EDTA drug product matrix has an EDTA concentration of between 10 μ M and 250 μ M. In some embodiments, the EDTA concentration is greater than or equal to 10 μ M, greater than or equal to 20 μ M, greater than or equal to 30 μ M, greater than or equal to 40 μ M, greater than or equal to 50 μ M, greater than or equal to 60 μ M, greater than or equal to 70 μ M, greater than or equal to 80 μ M, greater than or equal to 90 μ M, greater than or equal to 100 μ M, greater than or equal to 125 μ M, greater than or equal to 150 μ M, greater than or equal to 175 μ M, greater than or equal to 200 μ M, greater than or equal to 225 μ M, or greater than or equal to 250 μ M. In some embodiments, the EDTA concentration is less than or equal to 250 μ M, less than or equal to 150 μ M, less than or equal to 100 μ M, less than or equal to 90 μ M, less than or equal to 80 μ M, less than or equal to 70 μ M, less than or equal to 60 μ M, less than or equal to 50 μ M, less than or equal to 40 μ M, less than or equal to 30 μ M, less than or equal to 20 μ M, or less than or equal to 10 μ M. In some embodiments, the concentration of the EDTA in the EDTA drug product matrix is 50 μ M. Other combinations are also possible in some embodiments.

- 18 -

In some embodiments, an EDTA drug product matrix has an EDTA concentration of between 10 μ M and 250 μ M, between 20 μ M and 200 μ M, between 25 μ M and 150 μ M, between 30 μ M and 90 μ M, between 35 μ M and 75 μ M, between 40 μ M and 60 μ M, or between 45 μ M and 55 μ M. Other ranges are also possible in some embodiments.

5 In some embodiments, an EDTA drug product matrix has an EDTA concentration of between 10 μ M and 100 μ M, between 20 μ M and 80 μ M, or between 30 μ M and 70 μ M. In some embodiments, an EDTA drug product matrix has an EDTA concentration of at least 10 μ M, at least 20 μ M, at least 25 μ M, at least 30 μ M, at least 35 μ M, at least 40 μ M, at least 45 μ M, at least 50 μ M, at least 55 μ M, at least 60 μ M, at least 75 μ M, or at least 100 μ M. In some
10 embodiments, the EDTA drug product matrix has an EDTA concentration of 50 μ M.

In some embodiments, an EDTA drug product matrix has a pH value of between 6.0 to 8.5 (e.g., 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, or 8.5). In some embodiments, the pH is greater than or equal to 6.0, greater than or equal to 6.1, greater than or equal to 6.2, greater than or equal to 6.3, greater than
15 or equal to 6.4, greater than or equal to 6.6, greater than or equal to 6.7, greater than or equal to 6.8, greater than or equal to 6.9, greater than or equal to 7.0, greater than or equal to 7.1, greater than or equal to 7.2, greater than or equal to 7.3, greater than or equal to 7.4, greater than or equal to 7.5, greater than or equal to 7.6, greater than or equal to 7.7, greater than or equal to 7.8, greater than or equal to 7.9, greater than or equal to 8.0, greater than or equal to 8.1, greater than
20 or equal to 8.2, greater than or equal to 8.3, greater than or equal to 8.4, or greater than or equal to 8.5. In some embodiments, the pH is less than or equal to 6.0, less than or equal to 6.1, less than or equal to 6.2, less than or equal to 6.3, less than or equal to 6.4, less than or equal to 6.5, less than or equal to 6.6, less than or equal to 6.7, less than or equal to 6.8, less than or equal to 6.9, less than or equal to 7.0, less than or equal to 7.1, less than or equal to 7.2, less than or equal
25 to 7.3, less than or equal to 7.4, less than or equal to 7.5, less than or equal to 7.6, less than or equal to 7.7, less than or equal to 7.8, less than or equal to 7.9, or less than or equal to 8.0. In some embodiments, an EDTA drug product matrix has a pH value of between 7.0 to 8.0. In some embodiments, an EDTA drug product matrix has a pH of about 7.4.

In some embodiments, an EDTA drug product matrix comprises one or more salts. In
30 some embodiments, the EDTA drug product matrix comprises sodium chloride. In some embodiments, an EDTA drug product matrix comprises phosphate (e.g., an EDTA drug product

- 19 -

matrix comprising EDTA and phosphate-buffer saline (PBS)). In some embodiments, the concentration of the one or more salts is between 10 mM and 300 mM. In some embodiments, the concentration of the one or more salts is greater than or equal to 10 mM, greater than or equal to 20 mM, greater than or equal to 40 mM, greater than or equal to 80 mM, greater than or equal to 100 mM, greater than or equal to 120 mM, greater than or equal to 140 mM, greater than or equal to 160 mM, greater than or equal to 180 mM, greater than or equal to 200 mM, greater than or equal to 220 mM, greater than or equal to 240 mM, greater than or equal to 260 mM, greater than or equal to 280 mM, or greater than or equal to 300 mM. In some embodiments, the concentration of the one or more salts in the EDTA drug product matrix is less than or equal to 300 mM, less than or equal to 280 mM, less than or equal to 260 mM, less than or equal to 240 mM, less than or equal to 220 mM, less than or equal to 200 mM, less than or equal to 180 mM, less than or equal to 160 mM, less than or equal to 140 mM, less than or equal to 120 mM, less than or equal to 100 mM, less than or equal to 80 mM, less than or equal to 40 mM, less than or equal to 20 mM, or less than or equal to 10 mM.

15 In some embodiments, the concentration of the one or more salts in an EDTA drug product matrix is between 10 mM and 300 mM, between 20 mM and 280 mM, between 40 mM and 260 mM, between 80 mM and 240 mM, between 100 mM and 220 mM, between 120 mM and 200 mM, or between 140 mM and 180 mM. Other concentrations are also possible in other ranges and/or combinations in some embodiments.

20 In some embodiments, a drug product matrix comprising EDTA (e.g., an EDTA drug product matrix described herein) does not comprise at least one of: citrate; methionine; and tryptophan. In some embodiments, a drug product matrix comprising EDTA (e.g., an EDTA drug product matrix described herein) does not comprise more than one of: citrate; methionine; and tryptophan. In some embodiments, a drug product matrix comprising EDTA (e.g., an EDTA drug product matrix described herein) does not comprise citrate, methionine, and tryptophan. In some embodiments, a drug product matrix comprising EDTA (e.g., an EDTA drug product matrix described herein) does not comprise histidine.

30 In some embodiments, a drug product matrix is a methionine-containing drug product matrix (e.g., methionine drug product matrix). In some embodiments, a methionine drug product matrix has a methionine concentration of between 0.1 mM and 50 mM. In some embodiments, a methionine drug product matrix has a methionine concentration of between 5 mM and 50 mM.

- 20 -

In some embodiments, the methionine concentration is greater than or equal to 0.1 mM, greater than or equal to 0.5 mM, greater than or equal to 1.0 mM, greater than or equal to 1.5 mM, greater than or equal to 3 mM, greater than or equal to 5 mM, greater than or equal to 10 mM, greater than or equal to 20 mM, greater than or equal to 30 mM, greater than or equal to 40 mM, or greater than or equal to 50 mM. In some embodiments, the methionine concentration is less than or equal to 50 mM, less than or equal to 40 mM, less than or equal to 30 mM, less than or equal to 20 mM, less than or equal to 10 mM, or less than or equal to 5 mM. In some embodiments, the concentration of the methionine in the methionine drug product matrix drug product matrix is 10 mM. Other combinations are also possible in some embodiments.

10 In some embodiments, a methionine drug product matrix drug product matrix has a methionine concentration of between 0.1 mM and 5 mM, 5 mM and 50 mM, between 10 mM and 40 mM, or between 20 mM and 30 mM. Other ranges are also possible in some embodiments.

15 In some embodiments, a methionine drug product matrix has a methionine concentration of between 5 mM and 25 mM, between 10 mM and 20 mM, between 12 mM and 18 mM, or between 14 mM and 16 mM. In some embodiments, a methionine drug product matrix has a methionine concentration of at least 5 mM, at least 10 mM, at least 20 mM, at least 25 mM, or at least 30 mM. In some embodiments, the methionine drug product matrix has a methionine concentration of 10 mM.

20 In some embodiments, a methionine drug product matrix has a pH value of between 6.0 to 8.5 (e.g., 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, or 8.5). In some embodiments, the pH is greater than or equal to 6.0, greater than or equal to 6.1, greater than or equal to 6.2, greater than or equal to 6.3, greater than or equal to 6.4, greater than or equal to 6.6, greater than or equal to 6.7, greater than or equal to 6.8, greater than or equal to 6.9, greater than or equal to 7.0, greater than or equal to 7.1, greater than or equal to 7.2, greater than or equal to 7.3, greater than or equal to 7.4, greater than or equal to 7.5, greater than or equal to 7.6, greater than or equal to 7.7, greater than or equal to 7.8, greater than or equal to 7.9, greater than or equal to 8.0, greater than or equal to 8.1, greater than or equal to 8.2, greater than or equal to 8.3, greater than or equal to 8.4, or greater than or equal to 8.5. In some embodiments, the pH is less than or equal to 6.0, less than or equal to 6.1, less than or equal to 6.2, less than or equal to 6.3, less than or equal to 6.4, less than or equal to 6.5,

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- 21 -

less than or equal to 6.6, less than or equal to 6.7, less than or equal to 6.8, less than or equal to 6.9, less than or equal to 7.0, less than or equal to 7.1, less than or equal to 7.2, less than or equal to 7.3, less than or equal to 7.4, less than or equal to 7.5, less than or equal to 7.6, less than or equal to 7.7, less than or equal to 7.8, less than or equal to 7.9, or less than or equal to 8.0. In
5 some embodiments, a methionine drug product matrix has a pH value of between 7.0 to 8.0. In some embodiments, a methionine drug product matrix has a pH of about 7.4.

In some embodiments, a methionine drug product matrix comprises one or more salts. In some embodiments, the methionine drug product matrix comprises sodium chloride. In some
embodiments, a methionine drug product matrix comprises phosphate (e.g., a methionine drug
10 product matrix comprising methionine and phosphate-buffer saline (PBS)). In some embodiments, the concentration of the one or more salts is between 10 mM and 300 mM. In some embodiments, the concentration of the one or more salts is greater than or equal to 10 mM, greater than or equal to 20 mM, greater than or equal to 40 mM, greater than or equal to 80 mM, greater than or equal to 100 mM, greater than or equal to 120 mM, greater than or equal to 140
15 mM, greater than or equal to 160 mM, greater than or equal to 180 mM, greater than or equal to 200 mM, greater than or equal to 220 mM, greater than or equal to 240 mM, greater than or equal to 260 mM, greater than or equal to 280 mM, or greater than or equal to 300 mM. In some embodiments, the concentration of the one or more salts in the methionine drug product matrix is less than or equal to 300 mM, less than or equal to 280 mM, less than or equal to 260 mM,
20 less than or equal to 240 mM, less than or equal to 220 mM, less than or equal to 200 mM, less than or equal to 180 mM, less than or equal to 160 mM, less than or equal to 140 mM, less than or equal to 120 mM, less than or equal to 100 mM, less than or equal to 80 mM, less than or equal to 40 mM, less than or equal to 20 mM, or less than or equal to 10 mM.

In some embodiments, the concentration of the one or more salts in a methionine drug
25 product matrix is between 10 mM and 300 mM, between 20 mM and 280 mM, between 40 mM and 260 mM, between 80 mM and 240 mM, between 100 mM and 220 mM, between 120 mM and 200 mM, or between 140 mM and 180 mM. Other concentrations are also possible in other ranges and/or combinations in some embodiments.

In some embodiments, a drug product matrix comprising methionine (e.g., a methionine
30 drug product matrix described herein) does not comprise at least one of: citrate; EDTA; and tryptophan. In some embodiments, a drug product matrix comprising methionine (e.g., a

- 22 -

methionine drug product matrix described herein) does not comprise more than one of: citrate; EDTA; and tryptophan. In some embodiments, a drug product matrix comprising methionine (e.g., a methionine drug product matrix described herein) does not comprise citrate, EDTA, and tryptophan. In some embodiments, a drug product matrix comprising methionine (e.g., a
5 methionine drug product matrix described herein) does not comprise histidine.

In some embodiments, a drug product matrix is a tryptophan-containing drug product matrix (e.g., tryptophan drug product matrix). In some embodiments, a tryptophan drug product matrix has a tryptophan concentration of between 0.1 mM to 5 mM, or 5 mM and 50 mM. In some embodiments, the tryptophan concentration is greater than or equal to 0.1 mM, greater
10 than or equal to 0.5 mM, greater than or equal to 1.0 mM, greater than or equal to 1.5 mM, greater than or equal to 3 mM, greater than or equal to 5 mM, greater than or equal to 10 mM, greater than or equal to 20 mM, greater than or equal to 30 mM, greater than or equal to 40 mM, or greater than or equal to 50 mM. In some embodiments, the tryptophan concentration is less than or equal to 50 mM, less than or equal to 40 mM, less than or equal to 30 mM, less than or
15 equal to 20 mM, less than or equal to 10 mM, or less than or equal to 5 mM. In some embodiments, the concentration of the tryptophan in the tryptophan drug product matrix is 10 mM. Other combinations are also possible in some embodiments.

In some embodiments, a tryptophan drug product matrix has a tryptophan concentration of between 5 mM and 50 mM, between 10 mM and 40 mM, or between 20 mM and 30 mM.
20 Other ranges are also possible in some embodiments.

In some embodiments, a tryptophan drug product matrix has a tryptophan concentration of between 5 mM and 25 mM, between 10 mM and 20 mM, between 12 mM and 18 mM, or between 14 mM and 16 mM. In some embodiments, a tryptophan drug product matrix has a tryptophan concentration of at least 5 mM, at least 10 mM, at least 20 mM, at least 25 mM, or at
25 least 30 mM. In some embodiments, the tryptophan drug product matrix has a tryptophan concentration of 10 mM.

In some embodiments, a tryptophan drug product matrix has a pH value of between 6.0 to 8.5 (e.g., 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, or 8.5). In some embodiments, the pH is greater than or equal to 6.0,
30 greater than or equal to 6.1, greater than or equal to 6.2, greater than or equal to 6.3, greater than or equal to 6.4, greater than or equal to 6.6, greater than or equal to 6.7, greater than or equal to

- 23 -

6.8, greater than or equal to 6.9, greater than or equal to 7.0, greater than or equal to 7.1, greater than or equal to 7.2, greater than or equal to 7.3, greater than or equal to 7.4, greater than or equal to 7.5, greater than or equal to 7.6, greater than or equal to 7.7, greater than or equal to 7.8, greater than or equal to 7.9, greater than or equal to 8.0, greater than or equal to 8.1, greater than
5 or equal to 8.2, greater than or equal to 8.3, greater than or equal to 8.4, or greater than or equal to 8.5. In some embodiments, the pH is less than or equal to 6.0, less than or equal to 6.1, less than or equal to 6.2, less than or equal to 6.3, less than or equal to 6.4, less than or equal to 6.5, less than or equal to 6.6, less than or equal to 6.7, less than or equal to 6.8, less than or equal to 6.9, less than or equal to 7.0, less than or equal to 7.1, less than or equal to 7.2, less than or equal
10 to 7.3, less than or equal to 7.4, less than or equal to 7.5, less than or equal to 7.6, less than or equal to 7.7, less than or equal to 7.8, less than or equal to 7.9, or less than or equal to 8.0. In some embodiments, a tryptophan drug product matrix has a pH value of between 7.0 to 8.0. In some embodiments, a tryptophan drug product matrix has a pH of about 7.4.

In some embodiments, a tryptophan drug product matrix comprises one or more salts. In
15 some embodiments, the tryptophan drug product matrix comprises sodium chloride. In some embodiments, a tryptophan drug product matrix comprises phosphate (e.g., a tryptophan drug product matrix comprising tryptophan and phosphate-buffer saline (PBS)). In some embodiments, the concentration of the one or more salts is between 10 mM and 300 mM. In some embodiments, the concentration of the one or more salts is greater than or equal to 10 mM,
20 greater than or equal to 20 mM, greater than or equal to 40 mM, greater than or equal to 80 mM, greater than or equal to 100 mM, greater than or equal to 120 mM, greater than or equal to 140 mM, greater than or equal to 160 mM, greater than or equal to 180 mM, greater than or equal to 200 mM, greater than or equal to 220 mM, greater than or equal to 240 mM, greater than or equal to 260 mM, greater than or equal to 280 mM, or greater than or equal to 300 mM. In some
25 embodiments, the concentration of the one or more salts in the tryptophan drug product matrix is less than or equal to 300 mM, less than or equal to 280 mM, less than or equal to 260 mM, less than or equal to 240 mM, less than or equal to 220 mM, less than or equal to 200 mM, less than or equal to 180 mM, less than or equal to 160 mM, less than or equal to 140 mM, less than or equal to 120 mM, less than or equal to 100 mM, less than or equal to 80 mM, less than or equal
30 to 40 mM, less than or equal to 20 mM, or less than or equal to 10 mM.

- 24 -

In some embodiments, the concentration of the one or more salts in a tryptophan drug product matrix is between 10 mM and 300 mM, between 20 mM and 280 mM, between 40 mM and 260 mM, between 80 mM and 240 mM, between 100 mM and 220 mM, between 120 mM and 200 mM, or between 140 mM and 180 mM. Other concentrations are also possible in other ranges and/or combinations in some embodiments.

In some embodiments, a drug product matrix comprising tryptophan (e.g., a tryptophan drug product matrix described herein) does not comprise at least one of: citrate; EDTA; and methionine. In some embodiments, a tryptophan drug product matrix does not comprise citrate. In some embodiments, a methionine drug product matrix does not comprise EDTA. In some embodiments, a tryptophan drug product matrix does not comprise methionine. In some embodiments, a drug product matrix comprising tryptophan (e.g., a tryptophan drug product matrix described herein) does not comprise more than one of: citrate; EDTA; and methionine. In some embodiments, a drug product matrix comprising tryptophan (e.g., a tryptophan drug product matrix described herein) does not comprise citrate, EDTA, and methionine. In some embodiments, a drug product matrix comprising tryptophan (e.g., a tryptophan drug product matrix described herein) does not comprise histidine.

Composition Storage and Stability

Additional aspects of the present disclosure relate to storage of any one of the compositions described herein. For example, in some embodiments, the compositions are stored in a container such as a cartridge, prefilled syringe, or vial. In some embodiments, the container is a glass vial. In some embodiments, the container is a polycarbonate vial. It is known in the art, that all parenteral drugs must be stored in Type 1 glass (e.g., USP <660>, EP 3.2.1, ASTM E438) and meet requirements for hydrolytic resistance. However, the composition of the glass may vary significantly from manufacturer to manufacturer. Thus, in some embodiments, the vials may be obtained from a number of different manufacturers. In some embodiments, the vial comprises cyclic olefin polymer (herein "COP"). In some embodiments, the vial comprises glass. In some embodiments, the glass vial is a Corning Valor® glass vial. In some embodiments, the glass vial is a Schott BT5933 glass vial. In some embodiments, the glass vial is a Gerresheimer BT5974 glass vial. In some embodiments, the glass vial may comprise a coating. Exemplary coatings include, but are not limited to, ammonium sulfate, quartz (e.g.,

- 25 -

SiO_x), SiO₂, and the like. In some embodiments, the vials may have a coefficient of expansion (herein "COE") of 33 or 51, although other COEs are also contemplated herein. In some embodiments, the vials may have a volume of between 1 mL and 20 mL. In some embodiments, the vial has a volume of greater than or equal to 1 mL, greater than or equal to 5 mL, greater
5 than or equal to 10 mL, greater than or equal to 15 mL, or greater than or equal to 20 mL. In some embodiments, the vial has less than or equal to 20 mL, less than or equal to 15 mL, less than or equal to 10 mL, less than or equal to 5 mL, or less than or equal to 1 mL.

In some embodiments, the vials comprise a pharmaceutical rubber stopper or a cap. Any suitable pharmaceutical rubber stopper or cap known in the art may be used herein. In some
10 embodiments, the pharmaceutical rubber stopper or cap is provided with the vials (e.g., a Corning Valor® vial is supplied with its own rubber stopper). In some embodiments, a pharmaceutical rubber stopper is a VS5558 Serum stopper.

In some embodiments, the compositions are stored at a temperature above 4 °C. In some embodiments, the compositions are stored at a temperature between about 5 °C and about 30 °C,
15 between about 5 °C and about 70 °C, between about 10 °C and about 60 °C, between about 15 °C and about 55 °C, between about 20 °C and about 50 °C, between about 25 °C and about 45 °C, or between about 30 °C and 40 °C.

In some embodiments, the compositions are not cooled to less than or equal to 4 °C (e.g., temperature of most commercial refrigerators) or less than or equal to -20 °C (e.g., temperature
20 of most commercial freezers).

In some embodiments, the methods comprise storing the compositions are stored at a temperature between 5 °C and 25 °C. In some embodiments, the compositions are stored at a temperature between 5 °C and 30 °C. In some embodiments, the temperature is greater than or equal to 5 °C, greater than or equal to 10 °C, greater than or equal to 15 °C, greater than or equal
25 to 20 °C, greater than or equal to 25 °C, greater than or equal to 30 °C, greater than or equal to 35 °C, greater than or equal to 40 °C, greater than or equal to 45 °C, greater than or equal to 50 °C, greater than or equal to 55 °C, greater than or equal to 60 °C, greater than or equal to 65 °C, or greater than or equal to 70 °C. In some embodiments, the temperature is less than or equal to 70 °C, less than or equal to 65 °C, less than or equal to 60 °C, less than or equal to 55 °C, less
30 than or equal to 50 °C, less than or equal to 45 °C, less than or equal to 40 °C, less than or equal to 35 °C, less than or equal to 30 °C, less than or equal to 25 °C, less than or equal to 20 °C, less

- 26 -

than or equal to 10 °C, or less than or equal to 5 °C. Other combinations are also possible in some embodiments (e.g., greater than or equal to 5 °C and less than or equal to 30 °C or greater than or equal to 5 °C and less than or equal to 70 °C).

Aspects of the disclosure relate to compositions comprising LNPs comprising one or
5 more ionizable lipids and drug product matrices (e.g., citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, or tryptophan drug product matrix) disclosed elsewhere herein. In some embodiments, the one or more ionizable lipids is MC3 or DOTAP. In some embodiments, the drug product matrix is a citrate drug product matrix having a concentration ranging from about 5 mM to 25 mM of citrate (e.g., about 10 mM of citrate). In
10 some embodiments, the citrate drug product matrix has a pH ranging from about 6.5 to about 8.0 (e.g., a pH ranging from about 6.5 to about 7.5, such as a pH of about 7.0). In some embodiments, the drug product matrix is an EDTA drug product matrix having a concentration ranging from about 10 µM and 250 µM of EDTA (e.g., about 50 µM of EDTA). In some
15 embodiments, the EDTA drug product matrix has a pH ranging from about 6.5 to about 8.0 (e.g., a pH ranging from about 7.0 to about 8.0, such as a pH of about 7.4). In some embodiments, the drug product matrix is a methionine drug product matrix having a concentration ranging from about 5 mM to 25 mM of methionine (e.g., about 10 mM of methionine). In some embodiments, the methionine drug product matrix has a pH ranging from about 6.5 to about 8.0 (e.g., a pH
20 ranging from about 7.0 to about 8.0, such as a pH of about 7.4). In some embodiments, the drug product matrix is a tryptophan drug product matrix having a concentration ranging from about 5 mM to 25 mM of tryptophan (e.g., about 10 mM of tryptophan). In some embodiments, the tryptophan drug product matrix has a pH ranging from about 6.5 to about 8.0 (e.g., a pH ranging from about 7.0 to about 8.0, such as a pH of about 7.4).

In some embodiments, compositions comprising citrate drug product matrix LNP
25 compositions, EDTA drug product matrix LNP compositions, methionine drug product matrix LNP compositions, and/or tryptophan drug product matrix LNP compositions have at least a 200 % increase in the concentration of subvisible particles, relative to LNPs stored in phosphate buffer after 4 weeks of storage at 25 °C. As used herein, the term “subvisible particle” refers to particles less than or equal to 2 microns, for example, as determined by dynamic light scattering
30 (e.g., if LNP compositions stored in phosphate buffer had a subvisible particle concentration of 50,000 particle/mL and citrate drug product matrix LNP compositions, EDTA drug product

- 27 -

matrix LNP compositions, methionine drug product matrix LNP compositions, and/or tryptophan drug product matrix LNP compositions had a subvisible particle concentration of 150,000 particles/mL, the percent increase in would be $[150,000-50,000]/50,000 \times 100\% = 200\%$).

5 In some embodiments, the compositions comprising citrate drug product matrix LNP compositions, EDTA drug product matrix LNP compositions, methionine drug product matrix LNP compositions, and/or tryptophan drug product matrix LNP compositions have at least a 10 %, at least 20 %, at least 30 %, at least 40 %, at least 50 %, at least 60 %, at least 70 %, at least 80 %, at least 90 %, at least 100 %, at least 120 %, at least 140 %, at least 160 %, at least 180 %, 10 or at least 200 % increase in the concentration of subvisible particles, relative to LNPs stored in phosphate buffer (e.g., phosphate buffer without citrate, EDTA, methionine, or tryptophan) after 4 weeks of storage at 25 °C.

 In some embodiments, compositions comprising citrate drug product matrix LNP compositions, EDTA drug product matrix LNP compositions, methionine drug product matrix 15 LNP compositions, and/or tryptophan drug product matrix LNP compositions have an API concentration of at least 0.11 mg/mL or an encapsulation efficiency of at least 95 %, relative to LNPs stored in phosphate buffer (e.g., phosphate buffer without citrate, EDTA, methionine, or tryptophan) after 4 weeks of storage at 25 °C. In some embodiments, the compositions comprising excipient-containing (e.g., citrate, EDTA, methionine, or tryptophan containing) 20 LNP compositions have an siRNA concentration of at least 0.11 mg/mL or an encapsulation efficiency of at least 95 %, relative to LNPs stored in phosphate buffer after 4 weeks of storage at 25 °C.

 In some embodiments, the compositions comprising excipient-containing (e.g., citrate, EDTA, methionine, or tryptophan containing) LNP compositions undergo between 0.1 % and 2 25 % hydrolysis after 4 weeks of storage at 25 °C. In some embodiments, the compositions comprising excipient-containing (e.g., citrate, EDTA, methionine, or tryptophan containing) LNP compositions undergo between 0.1 % and 1 % oxidation after 4 weeks of storage at 25 °C. In some embodiments, the compositions comprising excipient-containing (e.g., citrate, EDTA, methionine, or tryptophan containing) LNP compositions contain between 95 % and 98.5 % 30 intact LNPs after 4 weeks of storage at 25 °C.

Methods

Aspects of the disclosure relate to one or more methods for producing LNP compositions disclosed herein. Exemplary LNPs include, but are not limited to, micelles, liposomes, lipoplexes, and solid lipid nanoparticles or derivatives thereof. LNPs may have any morphology and structure known in the art. For example, in some embodiments, LNPs are nanospheres, nanorods, nanochains, nanostars, nanoflowers, nanoreefs, nanowhiskers, nanofibers, and/or nanoboxes. In some embodiments, other morphologies and structures are also possible.

In some embodiments, the method comprises LNP compositions with varying ratios of one or more lipids. Any suitable ratio known in the art may be used to produce the LNPs disclosed herein. Those familiar with the art, will appreciate that the sum of the percent contribution from each lipid component cannot exceed 100% (e.g., mole %, weight %, mass %, volume %, etc.). For example, in some embodiments, the percent contribution of each lipid in the LNP composition is greater than or equal to 1%, greater than or equal to 5%, greater than or equal to 10%, greater than or equal to 15%, greater than or equal to 20%, greater than or equal to 25%, greater than or equal to 30%, greater than or equal to 35%, greater than or equal to 40%, greater than or equal to 45%, greater than or equal to 50%, greater than or equal to 55%, greater than or equal to 60%, greater than or equal to 65%, greater than or equal to 70%, greater than or equal to 75%, greater than or equal to 80%, greater than or equal to 85%, greater than or equal to 90%, greater than or equal to 95%, or greater than or equal to 100%. In some embodiments, the percent contribution (e.g., mole %) of each lipid in LNP composition is less than or equal to 100%, less than or equal to 95%, less than or equal to 90%, less than or equal to 85%, less than or equal to 80%, less than or equal to 75%, less than or equal to 70%, less than or equal to 65%, less than or equal to 60%, less than or equal to 55%, less than or equal to 50%, less than or equal to 45%, less than or equal to 40%, less than or equal to 35%, less than or equal to 30%, less than or equal to 25%, less than or equal to 20%, less than or equal to 15%, less than or equal to 10%, less than or equal to 5%, or less than or equal to 1%.

In some embodiments, the methods comprise formulating the LNP compositions using a drug product matrix or excipient-containing solution as described herein. In some embodiments, a drug product matrix or excipient-containing solution has a citrate, methionine, and/or tryptophan concentration of between 5 mM and 50 mM. In some embodiments, the concentration of the citrate, methionine, and/or tryptophan in the drug product matrix or

- 29 -

excipient-containing solution is 10 mM. In some embodiments, the drug product matrix or excipient-containing solution has a EDTA concentration of between 10 μ M and 250 μ M. In some embodiments, the concentration of the EDTA in the EDTA drug product matrix or excipient-containing solution is 50 μ M. Other combinations are also possible in some
5 embodiments.

 In some embodiments, a drug product matrix or excipient-containing solution (e.g., a citrate drug product matrix drug product matrix, EDTA drug product matrix, methionine drug product matrix, or a tryptophan drug product matrix) has a pH value of between 6.0 to 8.5 (e.g., 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1,
10 8.2, 8.3, 8.4, or 8.5). In some embodiments, a drug product matrix (e.g., a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, or a tryptophan drug product matrix) has a pH value of between 6.0 to 8.0 (e.g., 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0). In some embodiments, a drug product matrix (e.g., a citrate drug product matrix, EDTA drug product matrix, methionine drug
15 product matrix, or a tryptophan drug product matrix) has a pH value of between 6.5 to 8.0 (e.g., 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0). In some embodiments, a drug product matrix (e.g., a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, or a tryptophan drug product matrix) has a pH value of between 6.5 to 7.5 (e.g., 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, or 7.5). In some
20 embodiments, a drug product matrix (e.g., a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, or a tryptophan drug product matrix) has a pH value of between 7.0 to 8.0 (e.g., 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0). In some embodiments, a drug product matrix (e.g., a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, or a tryptophan drug product matrix) has a pH value of
25 between 7.0 to 7.5 (e.g., 7.0, 7.1, 7.2, 7.3, 7.4, or 7.5). In some embodiments, a drug product matrix (e.g., a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, or a tryptophan drug product matrix) has a pH value of about 7.0 or about 7.4.

 In some embodiments, a drug product matrix comprises one or more salts. In some embodiments, the drug product matrix comprises sodium chloride. In some embodiments, the
30 drug product matrix comprises phosphate (e.g., a drug product matrix comprising citrate, EDTA, methionine, and/or tryptophan as well as phosphate-buffered saline (PBS)). In some

- 30 -

embodiments, the concentration of the one or more salts is between 10 mM and 300 mM (e.g., about 10 mM, 20 mM, 40 mM, 80 mM, 100 mM, 120 mM, 140 mM, 160 mM, 180 mM, 200 mM, 220 mM, 240 mM, 260 mM, 280 mM, or 300 mM).

In some embodiments, the methods comprise formulating the LNP compositions using a citrate drug product matrix. In some embodiments, a citrate drug product matrix has a citrate concentration of between 5 mM and 50 mM. In some embodiments, the citrate concentration is greater than or equal to 5 mM, greater than or equal to 10 mM, greater than or equal to 20 mM, greater than or equal to 30 mM, greater than or equal to 40 mM, or greater than or equal to 50 mM. In some embodiments, the citrate concentration is less than or equal to 50 mM, less than or equal to 40 mM, less than or equal to 30 mM, less than or equal to 20 mM, less than or equal to 10 mM, or less than or equal to 5 mM. In some embodiments, the concentration of the citrate in the citrate drug product matrix is 10 mM. Other combinations are also possible in some embodiments.

In some embodiments, a citrate drug product matrix has a citrate concentration of between 5 mM and 25 mM, between 10 mM and 20 mM, between 12 mM and 18 mM, or between 14 mM and 16 mM. In some embodiments, a citrate drug product matrix drug product matrix has a citrate concentration of at least 5 mM, at least 10 mM, at least 20 mM, at least 25 mM, or at least 30 mM. In some embodiments, the citrate drug product matrix drug product matrix has a citrate concentration of 10 mM.

In some embodiments, a citrate drug product matrix has a citrate concentration of between 5 mM and 50 mM, between 10 mM and 40 mM, or between 20 mM and 30 mM. Other ranges are also possible in some embodiments.

In some embodiments, a citrate drug product matrix has a pH value of between 6.0 to 8.5 (e.g., 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, or 8.5). In some embodiments, the pH is greater than or equal to 6.0, greater than or equal to 6.1, greater than or equal to 6.2, greater than or equal to 6.3, greater than or equal to 6.4, greater than or equal to 6.6, greater than or equal to 6.7, greater than or equal to 6.8, greater than or equal to 6.9, greater than or equal to 7.0, greater than or equal to 7.1, greater than or equal to 7.2, greater than or equal to 7.3, greater than or equal to 7.4, greater than or equal to 7.5, greater than or equal to 7.6, greater than or equal to 7.7, greater than or equal to 7.8, greater than or equal to 7.9, greater than or equal to 8.0, greater than or equal to 8.1, greater than or

equal to 8.2, greater than or equal to 8.3, greater than or equal to 8.4, or greater than or equal to 8.5. In some embodiments, the pH is less than or equal to 6.0, less than or equal to 6.1, less than or equal to 6.2, less than or equal to 6.3, less than or equal to 6.4, less than or equal to 6.5, less than or equal to 6.6, less than or equal to 6.7, less than or equal to 6.8, less than or equal to 6.9, less than or equal to 7.0, less than or equal to 7.1, less than or equal to 7.2, less than or equal to 7.3, less than or equal to 7.4, less than or equal to 7.5, less than or equal to 7.6, less than or equal to 7.7, less than or equal to 7.8, less than or equal to 7.9, or less than or equal to 8.0. In some embodiments, a citrate drug product matrix has a pH value of between 6.5 to 7.5 or 7.0 to 7.5. In some embodiments, a citrate drug product matrix has a pH of about 7.0.

10 In some embodiments, a citrate drug product matrix comprises one or more salts. In some embodiments, the citrate drug product matrix comprises sodium chloride. In some embodiments, the concentration of the one or more salts is between 10 mM and 300 mM. In some embodiments, the concentration of the one or more salts is greater than or equal to 10 mM, greater than or equal to 20 mM, greater than or equal to 40 mM, greater than or equal to 80 mM, greater than or equal to 100 mM, greater than or equal to 120 mM, greater than or equal to 140 mM, greater than or equal to 160 mM, greater than or equal to 180 mM, greater than or equal to 200 mM, greater than or equal to 220 mM, greater than or equal to 240 mM, greater than or equal to 260 mM, greater than or equal to 280 mM, or greater than or equal to 300 mM. In some
15 embodiments, the concentration of the one or more salts in the citrate drug product matrix is less than or equal to 300 mM, less than or equal to 280 mM, less than or equal to 260 mM, less than or equal to 240 mM, less than or equal to 220 mM, less than or equal to 200 mM, less than or equal to 180 mM, less than or equal to 160 mM, less than or equal to 140 mM, less than or equal to 120 mM, less than or equal to 100 mM, less than or equal to 80 mM, less than or equal to 40 mM, less than or equal to 20 mM, or less than or equal to 10 mM.

25 In some embodiments, the concentration of the one or more salts in a citrate drug product matrix is between 10 mM and 300 mM, between 20 mM and 280 mM, between 40 mM and 260 mM, between 80 mM and 240 mM, between 100 mM and 220 mM, between 120 mM and 200 mM, or between 140 mM and 180 mM. Other concentrations are also possible in other ranges and/or combinations in some embodiments.

30 In some embodiments, the methods comprise formulating the LNP compositions using an EDTA drug product matrix. In some embodiments, an EDTA drug product matrix has an

- 32 -

EDTA concentration of between 10 μ M and 250 μ M. In some embodiments, the EDTA concentration is greater than or equal to 10 μ M, greater than or equal to 20 μ M, greater than or equal to 30 μ M, greater than or equal to 40 μ M, greater than or equal to 50 μ M, greater than or equal to 60 μ M, greater than or equal to 70 μ M, greater than or equal to 80 μ M, greater than or equal to 90 μ M, greater than or equal to 100 μ M, greater than or equal to 125 μ M, greater than or equal to 150 μ M, greater than or equal to 175 μ M, greater than or equal to 200 μ M, greater than or equal to 225 μ M, or greater than or equal to 250 μ M. In some embodiments, the EDTA concentration is less than or equal to 250 μ M, less than or equal to 150 μ M, less than or equal to 100 μ M, less than or equal to 90 μ M, less than or equal to 80 μ M, less than or equal to 70 μ M, less than or equal to 60 μ M, less than or equal to 50 μ M, less than or equal to 40 μ M, less than or equal to 30 μ M, less than or equal to 20 μ M, or less than or equal to 10 μ M. In some embodiments, the concentration of the EDTA in the EDTA drug product matrix is 50 μ M. Other combinations are also possible in some embodiments.

In some embodiments, an EDTA drug product matrix has an EDTA concentration of between 10 μ M and 250 μ M, between 20 μ M and 200 μ M, between 25 μ M and 150 μ M, between 30 μ M and 90 μ M, between 35 μ M and 75 μ M, between 40 μ M and 60 μ M, or between 45 μ M and 55 μ M. Other ranges are also possible in some embodiments.

In some embodiments, an EDTA drug product matrix has an EDTA concentration of between 10 μ M and 100 μ M, between 20 μ M and 80 μ M, or between 30 μ M and 70 μ M. In some embodiments, an EDTA drug product matrix has an EDTA concentration of at least 10 μ M, at least 20 μ M, at least 25 μ M, at least 30 μ M, at least 35 μ M, at least 40 μ M, at least 45 μ M, at least 50 μ M, at least 55 μ M, at least 60 μ M, at least 75 μ M, or at least 100 μ M. In some embodiments, the EDTA drug product matrix has an EDTA concentration of 50 μ M.

In some embodiments, an EDTA drug product matrix has a pH value of between 6.0 to 8.5 (e.g., 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, or 8.5). In some embodiments, the pH is greater than or equal to 6.0, greater than or equal to 6.1, greater than or equal to 6.2, greater than or equal to 6.3, greater than or equal to 6.4, greater than or equal to 6.6, greater than or equal to 6.7, greater than or equal to 6.8, greater than or equal to 6.9, greater than or equal to 7.0, greater than or equal to 7.1, greater than or equal to 7.2, greater than or equal to 7.3, greater than or equal to 7.4, greater than or equal to 7.5, greater than or equal to 7.6, greater than or equal to 7.7, greater than or equal to 7.8,

- 33 -

greater than or equal to 7.9, greater than or equal to 8.0, greater than or equal to 8.1, greater than or equal to 8.2, greater than or equal to 8.3, greater than or equal to 8.4, or greater than or equal to 8.5. In some embodiments, the pH is less than or equal to 6.0, less than or equal to 6.1, less than or equal to 6.2, less than or equal to 6.3, less than or equal to 6.4, less than or equal to 6.5, 5 less than or equal to 6.6, less than or equal to 6.7, less than or equal to 6.8, less than or equal to 6.9, less than or equal to 7.0, less than or equal to 7.1, less than or equal to 7.2, less than or equal to 7.3, less than or equal to 7.4, less than or equal to 7.5, less than or equal to 7.6, less than or equal to 7.7, less than or equal to 7.8, less than or equal to 7.9, or less than or equal to 8.0. In some embodiments, an EDTA drug product matrix has a pH value of between 7.0 to 8.0. In 10 some embodiments, an EDTA drug product matrix has a pH of about 7.4.

In some embodiments, an EDTA drug product matrix comprises one or more salts. In some embodiments, the EDTA drug product matrix comprises sodium chloride. In some embodiments, the EDTA drug product matrix comprise phosphate (e.g., a buffer comprising EDTA and phosphate-buffered saline (PBS)). In some embodiments, the concentration of the 15 one or more salts is between 10 mM and 300 mM. In some embodiments, the concentration of the one or more salts is greater than or equal to 10 mM, greater than or equal to 20 mM, greater than or equal to 40 mM, greater than or equal to 80 mM, greater than or equal to 100 mM, greater than or equal to 120 mM, greater than or equal to 140 mM, greater than or equal to 160 mM, greater than or equal to 180 mM, greater than or equal to 200 mM, greater than or equal to 220 mM, greater than or equal to 240 mM, greater than or equal to 260 mM, greater than or 20 equal to 280 mM, or greater than or equal to 300 mM. In some embodiments, the concentration of the one or more salts in the EDTA drug product matrix is less than or equal to 300 mM, less than or equal to 280 mM, less than or equal to 260 mM, less than or equal to 240 mM, less than or equal to 220 mM, less than or equal to 200 mM, less than or equal to 180 mM, less than or 25 equal to 160 mM, less than or equal to 140 mM, less than or equal to 120 mM, less than or equal to 100 mM, less than or equal to 80 mM, less than or equal to 40 mM, less than or equal to 20 mM, or less than or equal to 10 mM.

In some embodiments, the concentration of the one or more salts in an EDTA drug product matrix is between 10 mM and 300 mM, between 20 mM and 280 mM, between 40 mM 30 and 260 mM, between 80 mM and 240 mM, between 100 mM and 220 mM, between 120 mM

- 34 -

and 200 mM, or between 140 mM and 180 mM. Other concentrations are also possible in other ranges and/or combinations in some embodiments.

In some embodiments, the methods comprise formulating the LNP compositions using a methionine drug product matrix. In some embodiments, a methionine drug product matrix has a methionine concentration of between 5 mM and 50 mM. In some embodiments, the methionine concentration is greater than or equal to 5 mM, greater than or equal to 10 mM, greater than or equal to 20 mM, greater than or equal to 30 mM, greater than or equal to 40 mM, or greater than or equal to 50 mM. In some embodiments, the methionine concentration is less than or equal to 50 mM, less than or equal to 40 mM, less than or equal to 30 mM, less than or equal to 20 mM, less than or equal to 10 mM, or less than or equal to 5 mM. In some embodiments, the concentration of the methionine in the methionine drug product matrix is 10 mM. Other combinations are also possible in some embodiments.

In some embodiments, a methionine drug product matrix has a methionine concentration of between 5 mM and 25 mM, between 10 mM and 20 mM, between 12 mM and 18 mM, or between 14 mM and 16 mM. In some embodiments, a methionine drug product matrix has a methionine concentration of at least 5 mM, at least 10 mM, at least 20 mM, at least 25 mM, or at least 30 mM. In some embodiments, the methionine drug product matrix has a methionine concentration of 10 mM.

In some embodiments, a methionine drug product matrix has a methionine concentration of between 5 mM and 50 mM, between 10 mM and 40 mM, or between 20 mM and 30 mM. Other ranges are also possible in some embodiments.

In some embodiments, a methionine drug product matrix has a pH value of between 6.0 to 8.5 (e.g., 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, or 8.5). In some embodiments, the pH is greater than or equal to 6.0, greater than or equal to 6.1, greater than or equal to 6.2, greater than or equal to 6.3, greater than or equal to 6.4, greater than or equal to 6.6, greater than or equal to 6.7, greater than or equal to 6.8, greater than or equal to 6.9, greater than or equal to 7.0, greater than or equal to 7.1, greater than or equal to 7.2, greater than or equal to 7.3, greater than or equal to 7.4, greater than or equal to 7.5, greater than or equal to 7.6, greater than or equal to 7.7, greater than or equal to 7.8, greater than or equal to 7.9, greater than or equal to 8.0, greater than or equal to 8.1, greater than or equal to 8.2, greater than or equal to 8.3, greater than or equal to 8.4, or greater than or equal

- 35 -

to 8.5. In some embodiments, the pH is less than or equal to 6.0, less than or equal to 6.1, less than or equal to 6.2, less than or equal to 6.3, less than or equal to 6.4, less than or equal to 6.5, less than or equal to 6.6, less than or equal to 6.7, less than or equal to 6.8, less than or equal to 6.9, less than or equal to 7.0, less than or equal to 7.1, less than or equal to 7.2, less than or equal to 7.3, less than or equal to 7.4, less than or equal to 7.5, less than or equal to 7.6, less than or equal to 7.7, less than or equal to 7.8, less than or equal to 7.9, or less than or equal to 8.0. In some embodiments, a methionine drug product matrix has a pH value of between 7.0 to 8.0. In some embodiments, a methionine drug product matrix has a pH of about 7.4.

In some embodiments, a methionine drug product matrix comprises one or more salts. In some embodiments, the methionine drug product matrix comprises sodium chloride. In some embodiments, the methionine drug product matrix comprise phosphate (e.g., a buffer comprising methionine and phosphate-buffered saline (PBS)). In some embodiments, the concentration of the one or more salts is between 10 mM and 300 mM. In some embodiments, the concentration of the one or more salts is greater than or equal to 10 mM, greater than or equal to 20 mM, greater than or equal to 40 mM, greater than or equal to 80 mM, greater than or equal to 100 mM, greater than or equal to 120 mM, greater than or equal to 140 mM, greater than or equal to 160 mM, greater than or equal to 180 mM, greater than or equal to 200 mM, greater than or equal to 220 mM, greater than or equal to 240 mM, greater than or equal to 260 mM, greater than or equal to 280 mM, or greater than or equal to 300 mM. In some embodiments, the concentration of the one or more salts in the methionine drug product matrix is less than or equal to 300 mM, less than or equal to 280 mM, less than or equal to 260 mM, less than or equal to 240 mM, less than or equal to 220 mM, less than or equal to 200 mM, less than or equal to 180 mM, less than or equal to 160 mM, less than or equal to 140 mM, less than or equal to 120 mM, less than or equal to 100 mM, less than or equal to 80 mM, less than or equal to 40 mM, less than or equal to 20 mM, or less than or equal to 10 mM.

In some embodiments, the concentration of the one or more salts in a methionine drug product matrix is between 10 mM and 300 mM, between 20 mM and 280 mM, between 40 mM and 260 mM, between 80 mM and 240 mM, between 100 mM and 220 mM, between 120 mM and 200 mM, or between 140 mM and 180 mM. Other concentrations are also possible in other ranges and/or combinations in some embodiments.

- 36 -

In some embodiments, the methods comprise formulating the LNP compositions using a tryptophan drug product matrix. In some embodiments, a tryptophan drug product matrix has a tryptophan concentration of between 5 mM and 50 mM. In some embodiments, the tryptophan concentration is greater than or equal to 5 mM, greater than or equal to 10 mM, greater than or
5 equal to 20 mM, greater than or equal to 30 mM, greater than or equal to 40 mM, or greater than or equal to 50 mM. In some embodiments, the tryptophan concentration is less than or equal to 50 mM, less than or equal to 40 mM, less than or equal to 30 mM, less than or equal to 20 mM, less than or equal to 10 mM, or less than or equal to 5 mM. In some embodiments, the concentration of the tryptophan in the tryptophan drug product matrix is 10 mM. Other
10 combinations are also possible in some embodiments.

In some embodiments, a tryptophan drug product matrix has a tryptophan concentration of between 5 mM and 25 mM, between 10 mM and 20 mM, between 12 mM and 18 mM, or between 14 mM and 16 mM. In some embodiments, a tryptophan drug product matrix has a tryptophan concentration of at least 5 mM, at least 10 mM, at least 20 mM, at least 25 mM, or at
15 least 30 mM. In some embodiments, the tryptophan drug product matrix has a tryptophan concentration of 10 mM.

In some embodiments, a tryptophan drug product matrix has a tryptophan concentration of between 5 mM and 50 mM, between 10 mM and 40 mM, or between 20 mM and 30 mM. Other ranges are also possible in some embodiments.

20 In some embodiments, a tryptophan drug product matrix has a pH value of between 6.0 to 8.5 (e.g., 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, or 8.5). In some embodiments, the pH is greater than or equal to 6.0, greater than or equal to 6.1, greater than or equal to 6.2, greater than or equal to 6.3, greater than or equal to 6.4, greater than or equal to 6.6, greater than or equal to 6.7, greater than or equal to
25 6.8, greater than or equal to 6.9, greater than or equal to 7.0, greater than or equal to 7.1, greater than or equal to 7.2, greater than or equal to 7.3, greater than or equal to 7.4, greater than or equal to 7.5, greater than or equal to 7.6, greater than or equal to 7.7, greater than or equal to 7.8, greater than or equal to 7.9, greater than or equal to 8.0, greater than or equal to 8.1, greater than or equal to 8.2, greater than or equal to 8.3, greater than or equal to 8.4, or greater than or equal
30 to 8.5. In some embodiments, the pH is less than or equal to 6.0, less than or equal to 6.1, less than or equal to 6.2, less than or equal to 6.3, less than or equal to 6.4, less than or equal to 6.5,

- 37 -

less than or equal to 6.6, less than or equal to 6.7, less than or equal to 6.8, less than or equal to 6.9, less than or equal to 7.0, less than or equal to 7.1, less than or equal to 7.2, less than or equal to 7.3, less than or equal to 7.4, less than or equal to 7.5, less than or equal to 7.6, less than or equal to 7.7, less than or equal to 7.8, less than or equal to 7.9, or less than or equal to 8.0. In some embodiments, a tryptophan drug product matrix has a pH value of between 7.0 to 8.0. In some embodiments, a tryptophan drug product matrix has a pH of about 7.4.

In some embodiments, a tryptophan drug product matrix comprises one or more salts. In some embodiments, the tryptophan drug product matrix comprises sodium chloride. In some embodiments, the tryptophan drug product matrix comprise phosphate (e.g., a buffer comprising tryptophan and phosphate-buffered saline (PBS)). In some embodiments, the concentration of the one or more salts is between 10 mM and 300 mM. In some embodiments, the concentration of the one or more salts is greater than or equal to 10 mM, greater than or equal to 20 mM, greater than or equal to 40 mM, greater than or equal to 80 mM, greater than or equal to 100 mM, greater than or equal to 120 mM, greater than or equal to 140 mM, greater than or equal to 160 mM, greater than or equal to 180 mM, greater than or equal to 200 mM, greater than or equal to 220 mM, greater than or equal to 240 mM, greater than or equal to 260 mM, greater than or equal to 280 mM, or greater than or equal to 300 mM. In some embodiments, the concentration of the one or more salts in the tryptophan drug product matrix is less than or equal to 300 mM, less than or equal to 280 mM, less than or equal to 260 mM, less than or equal to 240 mM, less than or equal to 220 mM, less than or equal to 200 mM, less than or equal to 180 mM, less than or equal to 160 mM, less than or equal to 140 mM, less than or equal to 120 mM, less than or equal to 100 mM, less than or equal to 80 mM, less than or equal to 40 mM, less than or equal to 20 mM, or less than or equal to 10 mM.

In some embodiments, the concentration of the one or more salts in a tryptophan drug product matrix is between 10 mM and 300 mM, between 20 mM and 280 mM, between 40 mM and 260 mM, between 80 mM and 240 mM, between 100 mM and 220 mM, between 120 mM and 200 mM, or between 140 mM and 180 mM. Other concentrations are also possible in other ranges and/or combinations in some embodiments.

Aspects of the disclosure relate to methods for improving the chemical stability of a LNP pharmaceutical compositions, such as, for example, the compositions disclosed herein. In some embodiments, the method comprises obtaining an LNP pharmaceutical composition comprising

- 38 -

a non-citrate drug product matrix. The term “non-citrate” buffer (or non-citrate drug product matrix) as used herein refers to any buffer that does not contain citrate or other form of citric acid. In some embodiments, the method comprises obtaining an LNP pharmaceutical composition comprising a non-EDTA drug product matrix. The term “non-EDTA” buffer (or non-EDTA drug product matrix) as used herein refers to any buffer that does not contain EDTA. In some embodiments, the method comprises obtaining an LNP pharmaceutical composition comprising a non-methionine drug product matrix. The term “non-methionine” buffer (or non-methionine drug product matrix) as used herein refers to any buffer that does not contain the essential amino acid methionine. In some embodiments, the method comprises obtaining an LNP pharmaceutical composition comprising a non-tryptophan drug product matrix. The term “non-tryptophan” buffer (or non-tryptophan drug product matrix) as used herein refers to any buffer that does not contain the essential amino acid tryptophan. In some embodiments, the non-citrate drug product matrix, the non-EDTA drug product matrix, the non-methionine drug product matrix, and/or the non-tryptophan drug product matrix comprises a phosphate buffer (e.g., phosphate buffered saline). In some embodiments, the non-citrate drug product matrix, the non-EDTA drug product matrix, the non-methionine drug product matrix, and/or the non-tryptophan drug product matrix comprises phosphate buffered saline (PBS). However, other non-citrate drug product matrix, non-EDTA drug product matrix, non-methionine drug product matrix, and/or non-tryptophan drug product matrix are also possible, according to some embodiments. For example, in some embodiments, the non-citrate drug product matrix, the non-EDTA drug product matrix, the non-methionine drug product matrix, and/or the non-tryptophan drug product matrix is a bicarbonate buffer, a HEPES buffer, a MOPS buffer, a PBST buffer, a TBST buffer, a TE buffer, a TEN buffer or the like. Any method of preparation known in the art may be used to prepare any non-citrate drug product matrix, non-EDTA drug product matrix, non-methionine drug product matrix, and/or non-tryptophan drug product matrix contemplated herein, such as those described in Stoll et al., “Buffers: Principles and practice.” Meth. Enzymol. 1990. **182**, 24–38.

In some embodiments, the concentration of the conjugate acid-base pair used to create the non-citrate drug product matrix, the non-EDTA drug product matrix, the non-methionine drug product matrix, and/or the non-tryptophan drug product matrix is between 5 mM and 50 mM. In some embodiments, the concentration is greater than or equal to 5 mM, greater than or

- 39 -

equal to 10 mM, greater than or equal to 20 mM, greater than or equal to 30 mM, greater than or equal to 40 mM, or greater than or equal to 50 mM. In some embodiments, the concentration is less than or equal to 50 mM, less than or equal to 40 mM, less than or equal to 30 mM, less than or equal to 20 mM, less than or equal to 10 mM, or less than or equal to 5 mM. Other

5 combinations are also possible in some embodiments.

In some embodiments, the concentration of the conjugate acid-base pair used to create the non-citrate drug product matrix, the non-EDTA drug product matrix, the non-methionine drug product matrix, and/or the non-tryptophan drug product matrix is between 5 mM and 50 mM, between 10 mM and 40 mM, or between 20 mM and 30 mM. Other ranges are also

10 possible in some embodiments.

In some embodiments, a non-citrate drug product matrix, non-EDTA drug product matrix, non-methionine drug product matrix, and/or non-tryptophan drug product matrix has a pH value of between 5.8 to 6.5. In some embodiments, the pH is greater than or equal to 5.8, greater than or equal to 5.9, greater than or equal to 6.0, greater than or equal to 6.1, greater than or equal to 6.2, greater than or equal to 6.3, greater than or equal to 6.4, or greater than or equal to 6.5. In some embodiments, the pH is less than or equal to 6.5, less than or equal to 6.4, less than or equal to 6.3, less than or equal to 6.2, less than or equal to 6.1, less than or equal to 6.0, less than or equal to 5.9, or less than or equal to 5.8.

In some embodiments, a non-citrate drug product matrix, non-EDTA drug product matrix, non-methionine drug product matrix, and/or non-tryptophan drug product matrix has a pH value of between 5.8 to 6.5. In some embodiments, the non-citrate drug product matrix, the non-EDTA drug product matrix, the non-methionine drug product matrix, and/or the non-tryptophan drug product matrix has a pH value of between 5.8 to 6.4, between 5.8 and 6.3, between 5.8 and 6.2, between 5.8 and 6.1, between 5.8 and 6.0, or between 5.8 and 5.9. In some embodiments, the non-citrate drug product matrix, the non-EDTA drug product matrix, the non-methionine drug product matrix, and/or the non-tryptophan drug product matrix has a pH value of between 5.9 and 6.4, between 6.0 and 6.3, or between 6.1 and 6.2. In some embodiments, the non-citrate drug product matrix, the non-EDTA drug product matrix, the non-methionine drug product matrix, and/or the non-tryptophan drug product matrix has a pH value of between 5.9 and 6.4, between 6.0 and 6.4, between 6.1 and 6.4, between 6.2 and 6.4, or between 6.3 and 6.4.

- 40 -

In some embodiments, a non-citrate drug product matrix, non-EDTA drug product matrix, non-methionine drug product matrix, and/or non-tryptophan drug product matrix has a pH value of between 6.5 to 8.5. In some embodiments, the pH is greater than or equal to 6.5, greater than or equal to 6.6, greater than or equal to 6.7, greater than or equal to 6.8, greater than or equal to 6.9, greater than or equal to 7.0, greater than or equal to 7.1, greater than or equal to 7.2, greater than or equal to 7.3, greater than or equal to 7.4, greater than or equal to 7.5, greater than or equal to 7.6, greater than or equal to 7.7, greater than or equal to 7.8, greater than or equal to 7.9, greater than or equal to 8.0, greater than or equal to 8.1, greater than or equal to 8.2, greater than or equal to 8.3, greater than or equal to 8.4, or greater than or equal to 8.5. In some embodiments, the pH is less than or equal to 6.5, less than or equal to 6.6, less than or equal to 6.7, less than or equal to 6.8, less than or equal to 6.9, less than or equal to 7.0, less than or equal to 7.1, less than or equal to 7.2, less than or equal to 7.3, less than or equal to 7.4, less than or equal to 7.5, less than or equal to 7.6, less than or equal to 7.7, less than or equal to 7.8, less than or equal to 7.9, less than or equal to 8.0, less than or equal to 8.1, less than or equal to 8.2, less than or equal to 8.3, less than or equal to 8.4, or less than or equal to 8.5.

In some embodiments, a non-citrate drug product matrix, non-EDTA drug product matrix, non-methionine drug product matrix, and/or non-tryptophan drug product matrix has a pH value of between 6.5 to 8.5. In some embodiments, the non-citrate drug product matrix, the non-EDTA drug product matrix, the non-methionine drug product matrix, and/or the non-tryptophan drug product matrix has a pH value of between 6.5 and 8.5, between 6.5 and 8.4, between 6.5 and 8.3, between 6.5 and 8.2, between 6.5 and 8.1, between 6.5 and 8.0, between 6.5 and 7.9, between 6.5 and 7.8, between 6.5 and 7.7, between 6.5 and 7.6, between 6.5 and 7.5, between 6.5 and 7.4, between 6.5 and 7.3, between 6.5 and 7.2, between 6.5 and 7.1, between 6.5 and 7.0, between 6.5 and 6.9, between 6.5 and 6.8, between 6.5 and 6.7, or between 6.5 and 6.6. In some embodiments, the non-citrate drug product matrix, the non-EDTA drug product matrix, the non-methionine drug product matrix, and/or the non-tryptophan drug product matrix has a pH value of between 6.5 and 7.5, between 6.6 and 7.4, between 6.7 and 7.3, between 6.8 and 7.2, or between 6.9 and 7.1.

In some embodiments, the non-citrate drug product matrix, the non-EDTA drug product matrix, the non-methionine drug product matrix, and/or the non-tryptophan drug product matrix comprises one or more salts. In some embodiments, the non-citrate drug product matrix, the

- 41 -

non-EDTA drug product matrix, the non-methionine drug product matrix, and/or the non-tryptophan drug product matrix comprises sodium chloride. In some embodiments, the non-citrate drug product matrix, the non-EDTA drug product matrix, the non-methionine drug product matrix, and/or the non-tryptophan drug product matrix comprises phosphate (e.g.,
5 phosphate-buffered saline (PBS)). In some embodiments, the concentration of the one or more salts is between 10 mM and 300 mM. In some embodiments, the concentration of the one or more salts is greater than or equal to 10 mM, greater than or equal to 20 mM, greater than or equal to 40 mM, greater than or equal to 80 mM, greater than or equal to 100 mM, greater than or equal to 120 mM, greater than or equal to 140 mM, greater than or equal to 160 mM, greater
10 than or equal to 180 mM, greater than or equal to 200 mM, greater than or equal to 220 mM, greater than or equal to 240 mM, greater than or equal to 260 mM, greater than or equal to 280 mM, or greater than or equal to 300 mM. In some embodiments, the concentration of the one or more salts in the non-citrate drug product matrix, the non-EDTA drug product matrix, the non-methionine drug product matrix, and/or the non-tryptophan drug product matrix is less than or
15 equal to 300 mM, less than or equal to 280 mM, less than or equal to 260 mM, less than or equal to 240 mM, less than or equal to 220 mM, less than or equal to 200 mM, less than or equal to 180 mM, less than or equal to 160 mM, less than or equal to 140 mM, less than or equal to 120 mM, less than or equal to 100 mM, less than or equal to 80 mM, less than or equal to 40 mM, less than or equal to 20 mM, or less than or equal to 10 mM.

20 In some embodiments, the non-citrate drug product matrix, the non-EDTA drug product matrix, the non-methionine drug product matrix, and/or the non-tryptophan drug product matrix comprises salts at a concentration of between 10 mM and 300 mM, between 20 mM and 280 mM, between 40 mM and 260 mM, between 80 mM and 240 mM, between 100 mM and 220 mM, between 120 mM and 200 mM, or between 140 mM and 180 mM. Other concentrations are
25 also possible in other ranges and/or combinations in some embodiments.

In some embodiments, methods described herein comprise performing a buffer exchange reaction to replace a first buffer with a second buffer. In some embodiments, the second buffer is buffer described herein (e.g., a buffer comprising citrate, EDTA, methionine, and/or tryptophan). In some embodiments, methods described herein comprise performing a buffer exchange
30 reaction to replace a non-citrate drug product matrix (the first buffer) with a citrate drug product matrix (the second buffer) to obtain a citrate-buffered LNP pharmaceutical composition. In some

- 42 -

embodiments, methods described herein comprise performing a buffer exchange reaction to replace a non-EDTA drug product matrix (the first buffer) with an EDTA drug product matrix (the second buffer) to obtain an EDTA-buffered LNP pharmaceutical composition. In some embodiments, methods described herein comprise performing a buffer exchange reaction to

5 replace a non-methionine drug product matrix (the first buffer) with a methionine drug product matrix (the second buffer) to obtain a methionine-buffered LNP pharmaceutical composition. In some embodiments, methods described herein comprise performing a buffer exchange reaction to replace a non-tryptophan drug product matrix (the first buffer) with a tryptophan drug product matrix (the second buffer) to obtain a tryptophan-buffered LNP pharmaceutical composition.

10 Any method for exchanging buffers known in the art may be used in for the buffer exchange reaction. Exemplary embodiments, include but are not limited to, dialysis, desalting, diafiltration, and tangential flow filtration.

In some embodiments, the buffer exchange reaction is performed using dialysis. Without wishing to be bound by theory, dialysis separates small molecules from large molecules by

15 allowing diffusion of only the small molecules through selectively permeable membranes. The solution to be dialyzed (e.g., non-citrate containing LNP composition, non-EDTA containing LNP composition, non-methionine containing LNP composition, or non-tryptophan containing LNP composition) is placed in a sealed dialysis membrane, of a particular molecular weight cutoff, and immersed in a selected buffer (e.g., citrate containing buffer, EDTA containing

20 buffer, methionine containing buffer, or tryptophan containing buffer). The molecules of a first buffer diffuse out of the dialysis bag while molecules of a second buffer diffuse into the bag (e.g., along their respective diffusion gradients). In some embodiments, a method comprising dialysis comprises diffusion of a non-citrate drug product matrix molecules out of a dialysis bag while citrate drug product matrix molecules diffuse in the dialysis bag. In some embodiments, a

25 method comprising dialysis comprises diffusion of a non-EDTA drug product matrix molecules out of a dialysis bag while EDTA drug product matrix molecules diffuse in the dialysis bag. In some embodiments, a method comprising dialysis comprises diffusion of a non-methionine drug product matrix molecules out of a dialysis bag while methionine drug product matrix molecules diffuse in the dialysis bag. In some embodiments, a method comprising dialysis comprises

30 diffusion of a non-tryptophan drug product matrix molecules out of a dialysis bag while tryptophan drug product matrix molecules diffuse in the dialysis bag. Once the solution reaches

- 43 -

equilibrium, the buffer exchange reaction stops. To restart the buffer exchange reaction, the dialysate must be replaced with fresh buffer (e.g., fresh citrate drug product matrix, fresh EDTA drug product matrix, fresh methionine drug product matrix, or fresh tryptophan drug product matrix) to re-establish the concentration gradients. This procedure is repeated until the non-citrate drug product matrix, the non-EDTA drug product matrix, the non-methionine drug product matrix, or the non-tryptophan drug product matrix is completely removed from the dialysis membrane.

In some embodiments, the buffer exchange reaction is performed using desalting. Without wishing to be bound by theory, desalting column are based on gel filtration chromatography techniques in which a solution containing the buffer to be exchanged (e.g., non-citrate drug product matrix, non-EDTA drug product matrix, non-methionine drug product matrix, or non-tryptophan drug product matrix) is added to a porous resin. Larger molecules in the solution (e.g., LNPs) flow around the porous resin via the void spaces, whereas smaller molecules (e.g., non-citrate salts, non-EDTA salts, non-methionine salts, or non-tryptophan salts) enter into the pores of the porous resin. By passing sample through a column resin bed of sufficient length and volume, macromolecules (e.g., LNPs) can be fully separated from small molecules that travel a greater distance through the pores of the resin bed. Varying the maximum effective pore size is known in the art to be the primary determinant of the size of molecules that can be separated for that particular resin (e.g., also referred to as the molecular weight cut off, MWCO). In some embodiments, any suitable MWCO known in the art may be used to perform the buffer exchange reaction as disclosed herein.

In some embodiments, desalting columns may perform the buffer exchange reaction either directly or indirectly. For example, in some embodiments, the desalting column may be used to separate a composition comprising LNPs and a non-citrate drug product matrix, non-EDTA drug product matrix, non-methionine drug product matrix, or non-tryptophan drug product matrix into LNPs in water and non-citrate drug product matrix, non-EDTA drug product matrix, non-methionine drug product matrix, or non-tryptophan drug product matrix in water. In this case, the desired buffer salts may be added directly to the aqueous solution of LNPs to yield the final composition comprising LNPs in a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix. Alternatively, the desalting column may be pre-equilibrated with the desired final buffer (e.g., citrate drug

- 44 -

product matrix, EDTA drug product matrix, methionine drug product matrix, or tryptophan drug product matrix). In this case, the LNPs mix with the citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, or tryptophan drug product matrix as they pass through the void space and elute from the column.

5 In some embodiments, desalting may be performed using a variety of formats, for example, chromatography columns, gravity-flow columns, chromatography cartridges, centrifuge columns, and centrifuge plates. Other formats are also possible in some embodiments.

 In some embodiments, the buffer exchange reaction is performed using diafiltration. Without wishing to be bound by theory, diafiltration is a process that simultaneously dilutes and
10 filters a solution. In this way, a composition (e.g., LNPs composition comprising a non-citrate drug product matrix, non-EDTA drug product matrix, non-methionine drug product matrix, or non-tryptophan drug product matrix) may be filtered to remove small molecules (e.g., salts) while continuously diluted with a desired diluent (e.g., a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, or tryptophan drug product matrix). As
15 described elsewhere herein, the MWCO of the filters (e.g., resins, dialysis tubing, etc.) play an important role in determining what salts may be removed and which are retained. Thus, in some embodiments, any suitable MWCO known in the art may be used to perform the buffer exchange reaction as disclosed herein.

 In some embodiments, the methods comprise storing the citrate drug product matrix,
20 EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP pharmaceutical composition to improve the stability of the composition. Without wishing to be bound by theory, it is believed that various lipid components of the LNPs may undergo degradation via ester hydrolysis or oxidation of unsaturated bonds when stored in non-citrate drug product matrix, non-EDTA drug product matrix, non-methionine drug product
25 matrix, and/or non-tryptophan drug product matrix (e.g., phosphate buffer) for prolonged times and at different temperatures. It has now been discovered that storing the LNP pharmaceutical compositions in a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix reduces the degradation (e.g., oxidation and/or hydrolysis) of one or more lipid components, relative to LNP pharmaceutical
30 compositions stored in a non-citrate drug product matrix, non-EDTA drug product matrix, non-

- 45 -

methionine drug product matrix, and/or non-tryptophan drug product matrix (e.g., phosphate buffer).

In some embodiments, the methods comprise storing LNP pharmaceutical compositions in vials. It is known in the art, that all parenteral drugs must be stored in Type 1 glass (e.g., USP
5 <660>, EP 3.2.1, ASTM E438) and meet requirements for hydrolytic resistance. However, the composition of the glass may vary significantly from manufacturer to manufacturer. Thus, in some embodiments, the vials may be obtained from a number of different manufacturers. In some embodiments, the vial comprises cyclic olefin polymer (herein "COP"). In some
10 embodiments, the vial comprises glass. In some embodiments, the glass vial is a Corning Valor® glass vial. In some embodiments, the glass vial is a Schott BT5933 glass vial. In some embodiments, the glass vial is a Gerresheimer BT5974 glass vial. In some embodiments, the glass vial may comprise a coating. Exemplary coatings include, but are not limited to, ammonium sulfate, quartz (e.g., SiOx), SiO₂, and the like. In some embodiments, the vials may have a coefficient of expansion (herein "COE") of 33 or 51, although other COEs are also
15 contemplated herein. In some embodiments, the vials may have a volume of between 1 mL and 20 mL. In some embodiments, the vial has a volume of greater than or equal to 1 mL, greater than or equal to 5 mL, greater than or equal to 10 mL, greater than or equal to 15 mL, or greater than or equal to 20 mL. In some embodiments, the vial has less than or equal to 20 mL, less than or equal to 15 mL, less than or equal to 10 mL, less than or equal to 5 mL, or less than or equal
20 to 1 mL.

In some embodiments, the vials comprise a pharmaceutical rubber stopper or a cap. Any suitable pharmaceutical rubber stopper or cap known in the art may be used herein. In some embodiments, the pharmaceutical rubber stopper or cap is provided with the vials (e.g., a Corning Valor® vial is supplied with its own rubber stopper). In some embodiments, a
25 pharmaceutical rubber stopper is a VS5558 Serum stopper.

In some embodiments, the methods comprise storing the LNP pharmaceutical compositions in a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix at a temperature above 4 °C. In some
embodiments, the compositions are stored at a temperature between about 5 °C and about 30 °C,
30 between about 5 °C and about 70 °C, between about 10 °C and about 60 °C, between about 15 °C

- 46 -

and about 55 °C, between about 20 °C and about 50 °C, between about 25 °C and about 45 °C, or between about 30 °C and 40 °C.

In some embodiments, the compositions are not cooled to less than or equal to 4 °C (e.g., temperature of most commercial refrigerators) or less than or equal to -20 °C (e.g., temperature
5 of most commercial freezers). In some embodiments, the compositions are frozen (e.g., stored below 0 °C, for example at -20 °C or -70 °C) and subsequently thawed.

In some embodiments, the methods comprise storing the compositions are stored at a temperature between 5 °C and 25 °C. In some embodiments, the compositions are stored at a temperature between 5 °C and 30 °C. In some embodiments, the temperature is greater than or
10 equal to 5 °C, greater than or equal to 10 °C, greater than or equal to 15 °C, greater than or equal to 20 °C, greater than or equal to 25 °C, greater than or equal to 30 °C, greater than or equal to 35 °C, greater than or equal to 40 °C, greater than or equal to 45 °C, greater than or equal to 50 °C, greater than or equal to 55 °C, greater than or equal to 60 °C, greater than or equal to 65 °C, or greater than or equal to 70 °C. In some embodiments, the temperature is less than or equal to
15 70 °C, less than or equal to 65 °C, less than or equal to 60 °C, less than or equal to 55 °C, less than or equal to 50 °C, less than or equal to 45 °C, less than or equal to 40 °C, less than or equal to 35 °C, less than or equal to 30 °C, less than or equal to 25 °C, less than or equal to 20 °C, less than or equal to 10 °C, or less than or equal to 5 °C. Other combinations are also possible in some embodiments (e.g., greater than or equal to 5 °C and less than or equal to 30 °C or greater
20 than or equal to 5 °C and less than or equal to 70 °C).

In some embodiments, the methods comprise adjusting the pH of a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix during storage to a pH value of between 6.5 to 8.5. In some embodiments, the pH is greater than or equal to 6.5, greater than or equal to 6.6, greater than or equal to 6.7, greater
25 than or equal to 6.8, greater than or equal to 6.9, greater than or equal to 7.0, greater than or equal to 7.1, greater than or equal to 7.2, greater than or equal to 7.3, greater than or equal to 7.4, greater than or equal to 7.5, greater than or equal to 7.6, greater than or equal to 7.7, greater than or equal to 7.8, greater than or equal to 7.9, or greater than or equal to 8.0.

In some embodiments, the methods comprise adjusting the pH of a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug
30 product matrix during storage to a pH value of between 6.5 to 8.5. In some embodiments, the

- 47 -

citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix has a pH value of between 6.5 and 8.5, between 6.5 and 8.4, between 6.5 and 8.3, between 6.5 and 8.2, between 6.5 and 8.1, between 6.5 and 8.0, between 6.5 and 7.9, between 6.5 and 7.8, between 6.5 and 7.7, between 6.5 and 7.6, or between 6.5 and 7.5. In some embodiments, the citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix has a pH value of between 6.5 and 7.5, between 6.6 and 7.4, between 6.7 and 7.3, between 6.8 and 7.2, or between 6.9 and 7.1.

In some embodiments, the methods comprise storing a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP pharmaceutical composition for 4 weeks at 25 °C wherein storing the composition increases the number of intact LNPs in a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP pharmaceutical composition, relative to a LNP pharmaceutical composition stored in a phosphate buffer. For example, in some embodiments, the percent increase of intact LNPs in the citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP pharmaceutical compositions, relative to LNP compositions stored in a phosphate buffer, is between about 0.5 % to 14 %. In some embodiments, the percent increase is greater than or equal to 0.5 %, greater than or equal to 1 %, greater than or equal to 2 %, greater than or equal to 3 %, greater than or equal to 4 %, greater than or equal to 5 %, greater than or equal to 6 %, greater than or equal to 7 %, greater than or equal to 8 %, greater than or equal to 9 %, greater than or equal to 10 %, greater than or equal to 11 %, greater than or equal to 12 %, greater than or equal to 13 %, or greater than or equal to 14 %, relative to LNP compositions stored in a phosphate buffer after 4 weeks at 25 °C. In some embodiments, the percent increase is less than or equal to 14 %, less than or equal to 14 %, less than or equal to 13 %, less than or equal to 12 %, less than or equal to 11 %, less than or equal to 10 %, less than or equal to 9 %, less than or equal to 8 %, less than or equal to 7 %, less than or equal to 6 %, less than or equal to 5 %, less than or equal to 4 %, less than or equal to 3 %, less than or equal to 2 %, less than or equal to 1 %, or less than or equal to 0.5 %, relative to LNP compositions stored in a phosphate buffer after 4 week at 25 °C.

- 48 -

In some embodiments, the percent increase of intact LNPs in a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP pharmaceutical composition, relative to a LNP composition stored in a phosphate buffer, is between 0.5 % and 14 %, between 1 % and 12 %, between 2 % and 10 %, 5 between 3 % and 8 %, or between 4 % and 6 %.

In some embodiments, the methods comprise storing a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP pharmaceutical composition, wherein storing the composition reduces the percent degradation (e.g., hydrolysis and/or oxidation) of one or more lipid components, relative to a 10 LNP compositions stored in a phosphate buffer. In some embodiments, the percent decrease in degradation is between 40 % and 85 %, relative to LNP compositions stored in phosphate buffers. In some embodiments, the percent decrease in degradation is greater than or equal to 40 %, greater than or equal to 45 %, greater than or equal to 50 %, greater than or equal to 55 %, greater than or equal to 60 %, greater than or equal to 65 %, greater than or equal to 70 %, 15 greater than or equal to 75 %, greater than or equal to 80 %, or greater than or equal to 85 %, relative to LNP compositions stored in phosphate buffers. In some embodiments, the percent degradation is less than or equal to 85 %, less than or equal to 80 %, less than or equal to 75 %, less than or equal to 70 %, less than or equal to 65 %, less than or equal to 60 %, less than or equal to 55 %, less than or equal to 50 %, less than or equal to 45 %, or less than or equal to 40 20 %, relative to LNP compositions stored in phosphate buffers.

In some embodiments, the methods comprise storing a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP pharmaceutical composition, wherein storing the composition reduces the percent increase in the percent hydrolysis by at least 85 %, relative to a LNP compositions stored in a 25 phosphate buffer after 4 weeks at 25 °C.

In some embodiments, percent increase in the percent hydrolysis is reduced by at least 5 %, at least 10 %, at least 20 %, at least 30 %, at least 40 %, at least 50 %, at least 60 %, at least 70 %, at least 80 %, or at least 85 %, relative to a LNP compositions stored in a phosphate buffer after 4 weeks at 25 °C.

30 In some embodiments, the methods comprise storing a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product

matrix LNP pharmaceutical composition, wherein storing the composition reduces the percent increase in the percent oxidation by at least 33 %, relative to a LNP compositions stored in a phosphate buffer.

In some embodiments, percent increase in the percent oxidation is reduced by at least 5
5 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 31 %, at least 32 %, or at least 33 %, relative to a LNP compositions stored in a phosphate buffer after 4 weeks at 25 °C.

In some embodiments, the methods disclosed herein increase the colloidal stability of citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP pharmaceutical compositions relative to LNP
10 pharmaceutical compositions stored in phosphate buffers. Without wishing to be bound by theory, it is believed that colloidal instability results in LNP aggregation which increases the LNP mean particle diameter, for example, as measured using dynamic light scattering and/or increases the turbidity of the compositions, for example, as measured using transmitted light.

In some embodiments, storing the citrate drug product matrix, EDTA drug product
15 matrix, methionine drug product matrix, and/or tryptophan drug product matrix containing pharmaceutical composition decreases the percent increase in the mean particle diameter of the composition, relative to LNP compositions stored in a phosphate buffer after 4 weeks at 25 °C. In some embodiments, storing the compositions decreases the percent increase in mean particle diameter by between 70 % and 100 %. In some embodiments, storing the compositions
20 decreases the percent increase in mean particle diameter by greater than or equal to 70 %, greater than or equal to 75 %, greater than or equal to 80 %, greater than or equal to 85 %, greater than or equal to 90 %, greater than or equal to 95 %, or greater than or equal to 100 %, relative to LNP compositions stored in a phosphate buffer after 4 weeks at 25 °C. In some
25 embodiments, storing the compositions decreases the percent increase in mean particle diameter by less than or equal to 100 %, less than or equal to 95 %, less than or equal to 90 %, less than or equal to 85 %, less than or equal to 80 %, less than or equal to 75 %, or less than or equal to 70 %, relative to LNP compositions stored in a phosphate buffer after 4 weeks at 25 °C.

In some embodiments, storing the citrate drug product matrix, EDTA drug product
matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP
30 pharmaceutical compositions decreases the percent increase in the mean particle diameter of the

- 50 -

composition by a between about 70 % and 100 %, between about 75% and 95%, or between about 80 % and 90 %.

In some embodiments, storing the citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP pharmaceutical compositions decreases turbidity of the composition by between 1 % and 99 %, relative to LNP compositions stored in a phosphate buffer after 4 weeks at 25 °C. In some embodiments, the percent increase in turbidity of the composition is decreased by greater than or equal to 5%, greater than or equal to 10%, greater than or equal to 50%, greater than or equal to 75%, or greater than or equal to 90%, relative to LNP compositions stored in a phosphate buffer after 4 weeks at 25 °C. In some embodiments, the percent increase in turbidity of the composition is less than or equal to 5 %, less than or equal to 10 %, less than or equal to 50 %, less than or equal to 75 %, or less than or equal to 90 %, relative to LNP compositions stored in a phosphate buffer after 4 weeks at 25 °C.

In some embodiments, storing the citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP pharmaceutical compositions decreases turbidity of the composition by between about 5 % and 10 %, between about 25 % and 75 %, or between about 50 % and 90 % relative to LNP compositions stored in a phosphate buffer after 4 weeks at 25 °C.

Aspects of the present disclosure further relate to methods for improving the encapsulation efficiency of an API within one or more of the citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP compositions disclosed herein. In some embodiments, method comprises preparing a first lipid composition in any one of the citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix disclosed herein. Any suitable lipid composition known in the art may be used to produce the first lipid composition. For example, in some embodiments, first lipid composition comprises Dlin-MC3-DMA, cholesterol, DSPC, and DMG-PEG-2k. In some embodiments, the first lipid composition comprises SM-102, cholesterol, DSPC, and DMG-PEG-2k. In some embodiments, first lipid composition comprises Alc-0315, cholesterol, DSPC, and DMG-PEG-2k.

In some embodiments, the lipids in the first lipid composition are mixed at various ratios. Any suitable ratio known in the art may be used to produce the LNPs disclosed herein. Those

familiar with the art, will appreciate that the sum of the percent contribution from each lipid component cannot exceed 100% (e.g., mole %, weight %, mass %, volume %, etc.). For example, in some embodiments, the mole percent of each lipid in the first lipid composition is greater than or equal to 1%, greater than or equal to 5%, greater than or equal to 10%, greater than or equal to 15%, greater than or equal to 20%, greater than or equal to 25%, greater than or equal to 30%, greater than or equal to 35%, greater than or equal to 40%, greater than or equal to 45%, greater than or equal to 50%, greater than or equal to 55%, greater than or equal to 60%, greater than or equal to 65%, greater than or equal to 70%, greater than or equal to 75%, greater than or equal to 80%, greater than or equal to 85%, greater than or equal to 90%, greater than or equal to 95%, or greater than or equal to 100%. In some embodiments, the mole percent of each lipid in the first lipid composition is less than or equal to 100%, less than or equal to 95%, less than or equal to 90%, less than or equal to 85%, less than or equal to 80%, less than or equal to 75%, less than or equal to 70%, less than or equal to 65%, less than or equal to 60%, less than or equal to 55%, less than or equal to 50%, less than or equal to 45%, less than or equal to 40%, less than or equal to 35%, less than or equal to 30%, less than or equal to 25%, less than or equal to 20%, less than or equal to 15%, less than or equal to 10%, less than or equal to 5%, or less than or equal to 1%.

In some embodiments, the methods comprise preparing a second lipid composition. Any suitable lipid composition known in the art may be used to produce the second lipid composition. In some embodiments, the second lipid composition comprises DOTAP. In some embodiments, the second lipid composition comprises Dlin-MC3-DMA. Other compositions are also possible in some embodiments.

In some embodiments, the first and/or second lipid composition has a lipid concentration of between 10 mM and 15 mM. In some embodiments, the concentration of the first and/or second lipid concentration is greater than or equal to 10 mM, greater than or equal to 10.5 mM, greater than or equal to 11 mM, greater than or equal to 11.5 mM, greater than or equal to 12 mM, greater than or equal to 12.5 mM, greater than or equal to 13 mM, greater than or equal to 13.5 mM, greater than or equal to 14 mM, greater than or equal to 14.5 mM, or greater than or equal to 15 mM.

In some embodiments, the concentration of the first and/or second lipid composition is between about 7.5 mM and 17.5 mM, between about 8.5 mM and 16.5 mM, between about 9.5

- 52 -

mM and 15.5 mM, between about 10.5 mM and 14.5 mM, or between about 11.5 mM and 13.5 mM.

In some embodiments, the methods comprise mixing an API (e.g., mRNA, siRNA, dsRNA, or miRNA) with the second lipid composition. In some embodiments, the API is an siRNA.

In some embodiments, the methods comprise mixing the one or more nucleic acid with the second lipid composition prior to mixing the second lipid composition with the first lipid composition. The concentration of the nucleic acid in the second lipid composition may be any concentration that has a therapeutic effect on a subject in need thereof. In some embodiments, the concentration of the one or more nucleic acids in the second lipid composition prior to mixing the second composition with the first lipid composition is greater than or equal to 50 micrograms/mL, greater than or equal to 75 micrograms/mL, greater than or equal to 100 micrograms/mL, greater than or equal to 120 micrograms/mL, greater than or equal to 150 micrograms/mL, greater than or equal to 175 micrograms/mL, or greater than or equal to 200 micrograms/mL. In some embodiments, the concentration of the one or more nucleic acids in the second lipid composition prior to mixing the second composition with the first lipid composition is less than or equal to 200 micrograms/mL, less than or equal to 175 micrograms/mL, less than or equal to 150 micrograms/mL, less than or equal to 120 micrograms/mL, less than or equal to 100 micrograms/mL, less than or equal to 75 micrograms/mL, or less than or equal to 50 micrograms/mL.

In some embodiments, the methods comprise mixing the one or more nucleic acid with the second lipid composition at a ratio of between 1:50 and 50:1 before mixing the second lipid composition with the first lipid composition. In some embodiments, the one or more nucleic acid is mixed with the second lipid composition at a ratio of greater than or equal to 1:1, greater than or equal to 5:1, greater than or equal to 10:1, greater than or equal to 20:1, greater than or equal to 30:1, greater than or equal to 40:1, or greater than or equal to 50:1 before mixing the first lipid composition with the second lipid composition (e.g., weight percent, mole percent, etc.). In some embodiments, the ratio of the first lipid composition to the second lipid composition is less than or equal to 50:1, less than or equal to 40:1, less than or equal to 30:1, less than or equal to 20:1, less than or equal to 10:1, less than or equal to 5:1, or less than or equal to 1:1 before mixing the first lipid composition with the second lipid composition (e.g., weight percent, mole

percent, etc.). Other combinations are possible in some embodiments. Other ranges are also possible in some embodiments.

In some embodiments, the methods comprise mixing the first lipid composition and second lipid composition. The first lipid composition may be added to the second lipid composition in any suitable ratio to form the LNPs disclosed herein. For example, in some embodiments, the ratio of the first lipid composition to the second lipid composition (e.g., 1st lipid composition:2nd lipid composition) is greater than or equal to 1:1, greater than or equal to 0.1:1, greater than or equal to 0.2:1, greater than or equal to 0.3:1, greater than or equal to 0.4:1, greater than or equal to 0.5:1, greater than or equal to 0.5:1, greater than or equal to 0.6:1, greater than or equal to 0.7:1, greater than or equal to 0.8:1, greater than or equal to 0.9:1, greater than or equal to 1:1, greater than or equal to 1:0.9, greater than or equal to 1:0.8, greater than or equal to 1:0.7, greater than or equal to 1:0.6, greater than or equal to 1:0.5, greater than or equal to 1:0.4, greater than or equal to 1:0.3, greater than or equal to 1:0.2, or greater than or equal to 1:0.1. In some embodiments, the ratio of the ratio of the first lipid composition to the second lipid composition is less than or equal to 1:0.1, less than or equal to 1:0.2, less than or equal to 1:0.3, less than or equal to 1:0.4, less than or equal to 1:0.5, less than or equal to 1:0.6, less than or equal to 1:0.7, less than or equal to 1:0.8, less than or equal to 1:0.9, less than or equal to 1:1, less than or equal to 0.9:1, less than or equal to 0.8:1, less than or equal to 0.7:1, less than or equal to 0.6:1, less than or equal to 0.5:1, less than or equal to 0.4:1, less than or equal to 0.3:1, less than or equal to 0.2:1, or less than or equal to 0.1:1.

In some embodiments, the ratio of the first lipid composition to the second lipid composition is greater than or equal to 1:1, greater than or equal to 5:1, greater than or equal to 10:1, greater than or equal to 20:1, greater than or equal to 30:1, greater than or equal to 40:1, or greater than or equal to 50:1. In some embodiments, the ratio of the first lipid composition to the second lipid composition is less than or equal to 50:1, less than or equal to 40:1, less than or equal to 30:1, less than or equal to 20:1, less than or equal to 10:1, less than or equal to 5:1, or less than or equal to 1:1. Other combinations are possible in some embodiments. Other ranges are also possible in some embodiments. In some embodiments, the first lipid composition and the second lipid composition comprise one or more of the same lipids (e.g., MC3, DOTAP, etc.).

In some embodiments, the methods described herein increase percent encapsulation efficiency of an API within citrate drug product matrix, EDTA drug product matrix, methionine

- 54 -

drug product matrix, and/or tryptophan drug product matrix LNP compositions, relative to LNP compositions stored in phosphate buffer, after 4 weeks at 25 °C. In some embodiments, the percent increase in encapsulation efficiency is between about 70 % and about 140 %, relative to a LNP composition stored in phosphate buffer after storage for 4 weeks at 25 °C.

5 In some embodiments, the percent increase in the percent encapsulation efficiency of an API within citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP compositions, is between 70 % and 140 %, between 75 % and 135 %, between 80 % and 130 %, between 85 % and 125 %, between 90 % and 120 %, between 95 % and 110 %, or between 100 % and 105 %, relative to LNP
10 compositions stored in phosphate buffer, after 4 weeks at 25 °C

 In some embodiments, the percent increase in the percent encapsulation efficiency is greater than or equal to 40 %, greater than or equal to 50 %, greater than or equal to 60 %, greater than or equal to 70 %, greater than or equal to 80 %, greater than or equal to 90 %, greater than or equal to 100 %, greater than or equal to 120%, or greater than or equal to 140 %, relative to LNP compositions stored in phosphate buffer, after 4 weeks at 25 °C. In some
15 embodiments, the percent increase in the percent encapsulation efficiency is less than or equal to 140 %, less than or equal to 120 %, less than or equal to 100 %, less than or equal to 90%, less than or equal to 80%, less than or equal to 70%, less than or equal to 60%, less than or equal to 50%, or less than or equal to 40%, relative to relative to LNP compositions stored in phosphate
20 buffer, after 4 weeks at 25 °C.

 In some embodiments, the methods described herein increase the relative percent of intact ionizable lipids within a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP compositions, relative to LNP compositions stored in phosphate buffer, after 4 weeks at 25 °C. Without
25 wishing to be bound by theory, it is generally believed that mixing the negatively charged RNAs (e.g., siRNA) with the ionizable lipids in the second lipid composition (described elsewhere herein) results in the formation of RNA-lipid complexes (e.g., electrostatic bonds). These complexes act to shield the lipids from the aqueous buffer and thus decreases the incidence of water mediated hydrolysis and oxidation.

30 In some embodiments, the percent increase in the relative percent of intact ionizable lipids in citrate drug product matrix, EDTA drug product matrix, methionine drug product

- 55 -

matrix, and/or tryptophan drug product matrix LNP compositions is between about 55 % and about 75 %, relative to LNP compositions stored in phosphate buffer after 4 weeks at 25 °C.

In some embodiments, the percent increase in the relative percent of intact ionizable lipid in citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP compositions is between about 55 % and 75 % or between about 60 % and 70 %, relative to LNP compositions stored in phosphate buffer after 4 weeks and 20 °C.

In some embodiments, the percent increase in the relative percent of intact ionizable lipids in citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP compositions is greater than or equal to 55%, greater than or equal to 60 %, greater than or equal to 65 %, greater than or equal to 70 %, or greater than or equal to 75 %, relative to LNP compositions stored in a phosphate buffer after 4 weeks at 25 °C. In some embodiments, the percent increase in the relative percent of intact ionizable lipids in citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP compositions is less than or equal to 75%, less than or equal to 70 %, less than or equal to 65%, less than or equal to 60%, or less than or equal to 55%, relative to LNP compositions stored in a phosphate buffer after 4 weeks at 25 °C.

In some embodiments, the methods described herein increase the relative percent of intact APIs within citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP compositions, relative to LNP compositions stored in phosphate buffer, after 4 weeks at 25 °C. In some embodiments, API comprises a siRNA comprising a sense strand (herein “SS-strands”) and an antisense strand (herein “AS-strands”).

In some embodiments, the percent increase in the relative percent of intact SS-strands in citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP compositions is at least 100 %, relative to LNP compositions stored in phosphate buffers (e.g., if the relative percent of intact SS-strands in a LNP composition comprising a phosphate buffer is ~40% after 4 weeks at 25 °C, and the relative percent of intact SS-strands in a LNP composition comprising a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product

- 56 -

matrix is ~80 %, under the same conditions, then the percent increase between the two is about ~100 %.

In some embodiments, the percent increase in the relative percent of intact SS-strands in citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or
5 tryptophan drug product matrix LNP compositions is at least 10 %, at least 20 %, at least 30 %, at least 40 %, at least 50 %, at least 60 %, at least 70 %, at least 80 %, at least 90 %, or at least 100 %, relative to LNP compositions stored in a phosphate buffer after 4 weeks at 25 °C.

In some embodiments, the percent increase in the relative percent of intact AS-strands in citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or
10 tryptophan drug product matrix LNP compositions is at least 275 %, relative to LNP compositions stored in phosphate buffers (e.g., if the relative percent of intact AS-strands in a LNP composition comprising a phosphate buffer is ~20% after 4 weeks at 25 °C, and the relative percent of intact SS-strands in a LNP composition comprising a citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product
15 matrix is ~75 %, under the same conditions, then the percent increase between the two is about ~275 %.

In some embodiments, the percent increase in the relative percent of intact AS-strands in citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix containing LNP compositions is at least 10 %, at least 20 %, at
20 least 30 %, at least 40 %, at least 50 %, at least 60 %, at least 70 %, at least 80 %, at least 90 %, at least 100 %, at least 120 %, at least 140 %, at least 160 %, at least 180 %, at least 200 %, at least 220 %, at least 240 %, at least 250 %, or at least 275 %, relative to LNP compositions stored in a phosphate buffer after 4 weeks at 25 °C.

In some embodiments, the methods described herein preserve the biological function of
25 APIs encapsulated within citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP compositions, relative to LNP compositions stored in phosphate buffers, after 4 weeks of storage at 25 °C. In some embodiments, the methods described herein increase the inhibitory concentration (e.g., IC₅₀) of siRNAs encapsulated within citrate drug product matrix, EDTA drug product matrix, methionine
30 drug product matrix, and/or tryptophan drug product matrix LNP compositions, relative to LNP compositions stored in phosphate buffers, after 4 weeks of storage at 25 °C. In some

- 57 -

embodiments, siRNAs encapsulated citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP compositions exhibit an inhibitory concentration of at least 5 picoM, at least 10 picoM, at least 20 picoM, at least 30 picoM, at least 40 picoM, or at least 50 picoM after storage for 4 weeks at 25 °C. In
5 some embodiments, siRNAs encapsulated within citrate drug product matrix, EDTA drug product matrix, methionine drug product matrix, and/or tryptophan drug product matrix LNP compositions exhibit an inhibitory concentration of at least 50 picoM. In some embodiments, siRNAs encapsulated within LNP compositions stored in phosphate buffers lack an inhibitory concentration after storage for 4 week at 25 °C (e.g., they are completely degraded and exhibit no
10 biological activity).

Aspects of the disclosure relate to compositions and methods useful for treating diseases or disorders associated with dysregulated expression of mRNA and/or the protein products they encode. In some embodiments, the compositions and methods described herein modulate the function, activity, and/or level the protein product encoded by the target mRNA by decreasing
15 target mRNA level and/or translation of that target mRNA in a cell or subject.

EXAMPLES

Example 1: Stability analyses of ionizable lipids

The stability of ionizable lipids, MC3 and DOTAP (FIG. 1), to hydrolytic and oxidative
20 degradation when stored in either phosphate buffers and/or tris acetate buffers was investigated.

Stock MC3 lipidic solutions were prepared by dissolving MC3 in an ethanolic solution to a final concentration of 4 mg/mL (e.g., 6 mM). Similarly, stock lipidic DOTAP solutions were prepared by dissolving DOTAP in an ethanolic solution to a final concentration of 4 mg/mL. Stock solutions of phosphate buffered saline (1x, pH 7.4) were prepared. Final lipidic solutions
25 were prepared by mixing PBS stock to the lipidic stock in a ratio of 3:1 (e.g., PBS stock:DOTAP in ethanol = 3:1; PBS stock:MC3 stock in ethanol = 3:1). Solutions were stored in either glass (BT5933) or polymer (COP) vials.

Dilute lipidic solutions were frozen at -70 °C before being thawed and stored at either 5 °C, 25 °C, or 40 °C for either 1 week, 2 weeks, or 4 weeks. At each timepoint, (e.g., after 1

week, 2 weeks, or 4 weeks) the lipid solution was analyzed via LC-MS to determine the extent of hydrolysis and/or oxidation present.

As shown in FIG. 2A, the % DOTAP that remained intact (e.g., was not degraded) decreased with increasing storage time (e.g., 1 week > 4 week) and was lowest for samples stored at 40 °C. Also, samples stored in BT5933 glass vials exhibited better stability compared to identical samples stored in COP vials when diluted in PBS buffer. LC-MS analysis indicated that the primary degradation pathway for DOTAP is hydrolysis (FIG. 3A) with oxidation only contributing to degradation at prolonged storage times and elevated storage temperature (FIG. 3B, see storage at 40 °C for 4 weeks).

FIG. 2B, shows the percentage (%) MC3 intact for the various test groups. The % MC3 remaining intact following storage at 5 °C and 25 °C, was lower than the % DOTAP for the same time points, suggesting that MC3 degrades faster than DOTAP under these storage conditions. As above, storage in the BT5933 glass vials improved stability relative to COP vials when diluted in PBS buffer. LC-MS analysis indicated that the primary degradation pathway for MC3 is oxidation at all conditions tested (see FIG. 3C). Data indicate hydrolysis is the major degradation mechanism for DOTAP (FIG. 3A).

Example 2: Influence of formulation on the chemical and colloidal stability of RNA-LNPs

Stock solutions of citrate, EDTA, methionine, or tryptophan were prepared and mixed with compositions comprising RNA-LNPs. The compositions were then adjusted to reach the desired pH and final concentrations of citrate, EDTA, methionine, or tryptophan prior to the compositions being stored at room temperature (RT) for four weeks.

The compositions were then analyzed to determine physical and chemical characteristics of RNA-LNPs. The data from these analyses is shown in Table 1 and FIGs. 4-7. The data for each buffer storage condition is shown as a value which is relative to a control buffer comprising the same constituents but lacking RNA-LNPs which was measured at the same time point during the four-week storage period.

In Table 1, the buffer conditions that the compositions were stored in are shown in the top row and the left-most column shows the physical and/or chemical parameter tested.

Table 1. Lipid formulation screening (4 weeks at RT) of siHPRT-LNPs

- 59 -

	10 mM Citrate, pH 7	1X PBS, pH 7.4, 50 μ M EDTA	1X PBS, pH 7.4, 10 mM Methionine	1X PBS, pH 7.4, 10 mM Tryptophan
Intact MC3 Lipid (>90%)	96	99	99	99
Intact Sense Strand (>80%)	96	97	97	97
Intact Antisense Strand (>80%)	88	91	91	82
Size (<100 nm)	83	64	63	67
Polydispersity Index (<0.30)	0.18	0.09	0.07	0.14
Encapsulation Efficiency (>90%)	94	96	97	94
Transmittance (>20%)	27	29	35	34
Clarity by visual (Yes (Y)/No (N))	Y	Y	Y	Y

Visual analyses of compositions stored in citrate, EDTA, methionine, or tryptophan drug product matrix did not indicate any changes in solution clarity (Table 1 and FIG. 4). RNA-LNP size and polydispersity index of compositions stored in citrate, EDTA, methionine, or tryptophan drug product matrix were reduced relative to controls after 2- and 4-weeks of storage at RT (Table 1 and FIG. 5). In addition, encapsulation efficiency and RNA concentrations were both increased in compositions stored in citrate, EDTA, methionine, or tryptophan drug product matrix relative to controls after 2- and 4-weeks of storage at RT (Table 1 and FIG. 6). Further analyses were used to measure the amount of dienone, an MC3 degradation byproduct, in the compositions after 2- and 4-weeks of storage at RT. Dienone levels were decreased in compositions stored in citrate, EDTA, methionine, or tryptophan drug product matrix relative to controls (FIG. 7). These results indicated that all compositions stored in citrate, EDTA, methionine, or tryptophan drug product matrix exhibited minimal changes over course of four weeks and suggested these buffers increased colloidal and chemical stability of RNA-LNP compositions.

EQUIVALENTS

- 60 -

While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements

specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B,” when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e., “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B

- 62 -

(and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
 - (i) a lipid nanoparticle (LNP) comprising one or more ionizable lipids; and
 - (ii) a drug product matrix, wherein the drug product matrix comprises:
 - (a) a concentration of citrate ranging from 5mM to 25 mM and a pH ranging from about 6.0 to about 8.5;
 - (b) a concentration of ethylenediaminetetraacetic acid (EDTA) ranging from 10 μ M to 250 μ M and a pH ranging from about 6.0 to about 8.5;
 - (c) a concentration of methionine ranging from 5mM to 25 mM and a pH ranging from about 6.0 to about 8.5; or
 - (d) a concentration of tryptophan ranging from 5mM to 25 mM and a pH ranging from about 6.0 to about 8.5.
2. The pharmaceutical composition of claim 1, wherein the concentration of citrate in (ii)(a), methionine in (ii)(c), or tryptophan in (ii)(d) ranges between 5 mM and 20 mM, or wherein the concentration of EDTA in (ii)(b) ranges between 25 μ M and 100 μ M.
3. The pharmaceutical composition of claim 1, wherein the concentration of citrate in (ii)(a), methionine in (ii)(c), or tryptophan in (ii)(d) is at least 5 mM, or wherein the concentration of EDTA in (ii)(b) is at least 25 μ M.
4. The pharmaceutical composition of any one of claims 1 to 3, wherein the concentration of citrate in (ii)(a), methionine in (ii)(c), or tryptophan in (ii)(d) is 10 mM, or the concentration of EDTA in (ii)(b) is 50 μ M.
5. The pharmaceutical composition of any one of claims 1 to 4, wherein the pH of the drug product matrix is about pH 6.9 to 7.5.

- 64 -

6. The pharmaceutical composition of any one of claims 1 to 4, wherein the pH of the drug product matrix is about pH 7.0 to 7.4.
7. The pharmaceutical composition of any one of claims 1 to 6, wherein the pH of the drug product matrix of (ii)(a) is about 7.0, or wherein pH of the drug product matrix of (ii)(b), (ii)(c), or (ii)(d) is about 7.4.
8. The pharmaceutical composition of any one of claims 1 to 7, wherein the drug product matrix further comprises one or more salts.
9. The pharmaceutical composition of claim 8, wherein the one or more salts comprises a sodium salt, optionally NaCl.
10. The pharmaceutical composition of any one of claims 1 to 9, wherein the drug product matrix of (ii)(b), (ii)(c), or (ii)(d) further comprises phosphate-buffered saline (PBS).
11. The pharmaceutical composition of any one of claims 1 to 10, wherein the one or more ionizable lipids comprises DLin-MC3-DMA (MC3) and/or Dioleoyl-3-trimethylammonium propane (DOTAP).
12. The pharmaceutical composition of claim 11, wherein the one or more ionizable lipid consists of MC3 or DOTAP.
13. The pharmaceutical composition of any one of claims 1 to 12, wherein the LNP comprises one or more nucleic acids.
14. The pharmaceutical composition of claim 13, wherein the one or more nucleic acids comprises RNA.
15. The pharmaceutical composition of claim 14, wherein the RNA is mRNA, siRNA, dsRNA, or miRNA.

- 65 -

16. The pharmaceutical composition of claim 14 or 15, wherein the RNA is siRNA.
17. The pharmaceutical composition of any one of claims 1 to 16, wherein the composition has not been refrigerated or frozen.
18. The pharmaceutical composition of any one of claims 1 to 16, wherein the composition is stored at a temperature above 4 °C.
19. The pharmaceutical composition of claim 18, wherein the temperature above 4 °C ranges from about 5 °C to about 30 °C.
20. A vial containing the pharmaceutical composition of any one of claims 1 to 19.
21. The vial of claim 20, wherein the vial is a glass vial.
22. A method for improving chemical stability of a lipid nanoparticle (LNP) pharmaceutical composition, the method comprising:
 - (i) obtaining
 - (a) a non-citrate-containing LNP pharmaceutical composition comprising a non-citrate drug product matrix,
 - (b) a non-ethylenediaminetetraacetic acid (EDTA)-containing LNP pharmaceutical composition comprising a non-EDTA drug product matrix,
 - (c) a non-methionine-containing pharmaceutical composition comprising a non-methionine drug product matrix, or
 - (d) a non-tryptophan-containing pharmaceutical composition comprising a non-tryptophan drug product matrix ; and
 - (ii) performing a buffer exchange reaction to replace

- 66 -

- (a) the non-citrate drug product matrix of (i)(a) with a citrate excipient solution having a pH between 6.0 and 8.5 to obtain a citrate-including LNP pharmaceutical composition,
- (b) the non-EDTA drug product matrix of (i)(b) with an EDTA excipient solution having a pH between 6.0 and 8.5 to obtain an EDTA-including LNP pharmaceutical composition,
- (c) the non-methionine drug product matrix of (i)(c) with a methionine excipient solution having a pH between 6.0 and 8.5 to obtain a methionine-including LNP pharmaceutical composition, or
- (d) the non-tryptophan drug product matrix of (i)(d) with a tryptophan excipient solution having a pH between 6.0 and 8.5 to obtain a tryptophan-including LNP pharmaceutical composition.

23. The method of claim 22, wherein the LNP pharmaceutical composition of (ii)(a), (ii)(b), (ii)(c), or (ii)(d) comprises one or more ionizable lipids comprising DLin-MC3-DMA (MC3) and/or Dioleoyl-3-trimethylammonium propane (DOTAP).

24. The method of claim 22 or 23, wherein the LNP pharmaceutical composition comprises one or more nucleic acids.

25. The method of claim 24, wherein the one or more nucleic acids comprises RNA.

26. The method of claim 25, wherein the RNA is mRNA, siRNA, dsRNA, or miRNA.

27. The method of claim 25 or 26, wherein the RNA is siRNA.

28. The method of any one of claims 22 to 27, wherein the citrate excipient solution of (ii)(a), the EDTA excipient solution of (ii)(b), the methionine excipient solution of (ii)(c), or the tryptophan excipient solution (ii)(d) has a pH of 6.9 to 7.5.

- 67 -

29. The method of any one of claims 22 to 28, wherein the citrate excipient solution of (ii)(a) has a pH of 7.0, the EDTA excipient solution of (ii)(b) has a pH of 7.4, the methionine excipient solution of (ii)(c) has a pH of 7.4, or the tryptophan excipient solution of (ii)(d) has a pH of 7.4.

30. The method of any one of claims 22 to 29, wherein the LNP pharmaceutical composition of (ii)(a) has a concentration of citrate ranging from about 5mM to about 25 mM, the LNP pharmaceutical composition of (ii)(b) has a concentration of EDTA ranging from about 10 μ M to about 250 μ M, the LNP pharmaceutical composition of (ii)(c) has a concentration of methionine ranging from about 5mM to about 25 mM, or the LNP pharmaceutical composition of (ii)(d) has a concentration of tryptophan ranging from about 5mM to about 25 mM.

31. The method of any one of claims 22 to 30, wherein the LNP pharmaceutical composition of (ii)(a) has a concentration of citrate ranging from about 5 mM to 20 mM, the LNP pharmaceutical composition of (ii)(b) has a concentration of EDTA ranging from about 25 μ M to 100 μ M, the LNP pharmaceutical composition of (ii)(c) has a concentration of methionine ranging from about 5 mM to 20 mM, or the LNP pharmaceutical composition of (ii)(d) has a concentration of tryptophan ranging from about 5 mM to 20 mM.

32. The method of any one of claims 22 to 29, wherein the concentration of citrate in the LNP pharmaceutical composition of (ii)(a) is at least 5 mM, the concentration of EDTA in LNP pharmaceutical composition of (ii)(b) is at least 25 μ M, the concentration of methionine in the LNP pharmaceutical composition of (ii)(c) is at least 10 mM, or the concentration of tryptophan in the LNP pharmaceutical composition of (ii)(d) is at least 5 mM.

33. The method of any one of claims 22 to 32, wherein the concentration of citrate in the LNP pharmaceutical composition of (ii)(a) is 10 mM, the concentration of EDTA in the LNP pharmaceutical composition of (ii)(b) is 50 μ M, the concentration of methionine in the LNP pharmaceutical composition of (ii)(c) is 10 mM, or the concentration of tryptophan in the LNP pharmaceutical composition of (ii)(d) is 10 mM.

- 68 -

34. The method of any one of claims 22 to 33, wherein the buffer exchange reaction comprises contacting the non-citrate-containing LNP pharmaceutical composition of (i)(a), the non-EDTA-containing LNP pharmaceutical composition of (i)(b), the non-methionine-containing LNP pharmaceutical composition of (i)(c), or the non-tryptophan-containing LNP pharmaceutical composition of (i)(d) to a de-salting column.

35. The method of any one of claims 22 to 34, wherein the buffer exchange reaction comprises contacting the non-citrate-containing LNP pharmaceutical composition of (i)(a), the non-EDTA-containing LNP pharmaceutical composition of (i)(b), the non-methionine-containing LNP pharmaceutical composition of (i)(c), or the non-tryptophan-containing LNP pharmaceutical composition of (i)(d) to a dialysis tube.

36. The method of any one of claims 22 to 35, wherein the buffer exchange reaction comprises collecting the LNP pharmaceutical composition of (ii)(a), (ii)(b), (ii)(c), or (ii)(d) in a vial.

37. The method of claim 36, wherein the vial is a glass vial.

38. The method of any one of claims 22 to 37, further comprising storing the LNP pharmaceutical composition of (ii)(a), (ii)(b), (ii)(c), or (ii)(d) at a temperature above 4 °C.

39. The method of claim 38, wherein the temperature above 4 °C ranges from about 5 °C to about 30 °C.

40. The method of any one of claims 22 to 39, wherein the LNP pharmaceutical composition of (ii)(a), (ii)(b), (ii)(c), or (ii)(d) comprises fewer hydrolyzed lipids relative to a LNP pharmaceutical composition stored in a phosphate buffer that does not contain citrate, EDTA, methionine, or tryptophan.

41. The method of any one of claims 22 to 40, wherein the LNP pharmaceutical composition of (ii)(a), (ii)(b), (ii)(c), or (ii)(d) comprises fewer oxidized lipids relative to a LNP

- 69 -

pharmaceutical composition stored in a phosphate buffer that does not contain citrate, EDTA, methionine, or tryptophan.

42. The method of any one of claims 22 to 41, wherein the LNP pharmaceutical composition of (ii)(a), (ii)(b), (ii)(c), or (ii)(d) comprises LNPs having increased colloidal stability relative to LNPs stored in a pharmaceutical composition comprising phosphate buffer that does not contain citrate, EDTA, methionine, or tryptophan.

43. A method for preparing a lipid nanoparticle (LNP) pharmaceutical composition, the method comprising:

- (i) preparing a first lipid composition comprising one or more lipids;
- (ii) preparing a second lipid composition comprising a siRNA and an ionizable lipid;
and
- (iii) mixing the first lipid composition and the second lipid composition,
wherein the first lipid composition and second lipid composition are prepared using
 - (a) a citrate excipient solution having a pH between 6.0 and 8.5 and a citrate concentration of between 5mM and 25 mM,
 - (b) an ethylenediaminetetraacetic acid (EDTA) excipient solution having a pH between 6.0 and 8.5 and an EDTA concentration of between 10 μ M and 250 μ M,
 - (c) a methionine excipient solution having a pH between 6.0 and 8.5 and a methionine concentration of between 5mM and 25 mM, or
 - (d) a tryptophan excipient solution having a pH between 6.0 and 8.5 and a tryptophan concentration of between 5mM and 25 mM.

44. A method for preparing a lipid nanoparticle (LNP) pharmaceutical composition, the method comprising:

- (i) obtaining a lipid composition comprising a siRNA and an ionizable lipid,
and;
- (ii) mixing the lipid composition with a drug product matrix selected from:

- 70 -

- (a) a citrate excipient solution having a concentration ranging from 5mM to 25 mM citrate and a pH ranging from about 6.0 to about 8.5;
- (b) an ethylenediaminetetraacetic acid (EDTA) excipient solution having a concentration ranging from 10 μ M to 250 μ M EDTA and a pH ranging from about 6.0 to about 8.5;
- (c) a methionine excipient solution having a concentration ranging from 5mM to 25 mM methionine and a pH ranging from about 6.0 to about 8.5; or
- (d) a tryptophan excipient solution having a concentration ranging from 5mM to 25 mM tryptophan and a pH ranging from about 6.0 to about 8.5.