Claims

1. A method of preparing an empty-lipid nanoparticle solution (empty-LNP solution) comprising an empty lipid nanoparticle (empty LNP), comprising:

(i) a mixing step, comprising mixing a lipid solution comprising an ionizable lipid, a phospholipid, a PEG lipid and a structural lipid with an aqueous buffer solution comprising a first buffering agent, thereby forming the empty-LNP solution comprising the empty LNP, wherein the empty LNP comprises from about 0.1 mol % to about 0.5 mol % of the PEG lipid,

wherein the empty-LNP solution comprises an acetate buffer and has a pH in the range of about 4.6 to about 6.0.

2. The method of claim 1, further comprising processing the empty-LNP solution.

3. The method of claim 2, wherein processing the empty-LNP solution further comprises pH adjusting.

4. The method of claim 3, wherein the pH adjusting comprises adding a second buffering agent.

5. The method of claim 3 or 4, wherein a residence time between the mixing and the processing step is in a range of about 1.0 milliseconds to about 60 minutes, about 2.0 milliseconds to about 30 minutes, about 3.0 milliseconds to about 15 minutes, about 4.0 milliseconds to about 10 minutes, about 5.0 milliseconds to about 5 minutes about 10.0 milliseconds to about 2 minutes, about 100.0 milliseconds to about 1.0 minute, or about 1000 milliseconds to about 1.0 minute.

6. The method of any one of claims 2-5, wherein processing the empty-LNP solution further comprises filtering.

7. The method of any one of claims 2-6, wherein the step of processing the empty-LNP solution further comprises diluting the empty-LNP solution.

8. The method of any one of claims 1-7, wherein the aqueous buffer solution has a pH in a range of from about 4.5 to about 6.5, from about 4.6 to about 6.0, from about 4.7 to about 5.75, from about 4.8 to about 5.5, or from about 4.9 to about 5.25.

9. The method of claim 8, wherein the aqueous buffer solution has a pH of about 5.0.

10. The method of any one of claims 1-9, wherein the empty-LNP solution has a pH in a range of from about 4.8 to about 5.8, from about 5.0 to about 5.75, or from about 5.0 to about 5.5.

11. The method of any one of claims 1-10, wherein the lipid solution further comprises a first organic solvent.

12. The method of any one of claims 1-11, wherein the first buffering agent comprises a first aqueous buffer solution.

13. The method of any one of claims 1-12, wherein the first aqueous buffer is selected from the group consisting of an acetate buffer, a citrate buffer, a phosphate buffer, and a tris buffer.

14. The method of any one of claims 1-13, wherein the empty LNP comprises
about 30-60 mol% ionizable lipid;
about 0-30 mol% phospholipid;
about 15-50 mol% structural lipid; and
about 0.1-0.5 mol% PEG lipid.

15. The method of any one of claims 1-13, further comprising:

(ii) a loading step, comprising mixing a nucleic acid solution comprising a nucleic acid with the empty-LNP solution, thereby forming a loaded-LNP solution comprising a loaded LNP.

16. The method of claim 14, further comprising processing the loaded-LNP solution, thereby forming a lipid nanoparticle formulation (LNP formulation).

17. The method of claim 15, wherein processing the loaded-LNP solution comprises a first adding step, comprising adding a polyethylene glycol lipid (PEG lipid) to the loaded LNP.

18. The method of any one of claim 15 or 16, wherein processing the loaded-LNP solution further comprises buffer exchanging.

19. The method of claim 17, wherein the buffer exchanging comprises addition of an aqueous buffer solution comprising a third buffering agent.

20. The method of any one of claims 15-18, wherein processing the loaded-LNP solution comprises a second adding step, comprising adding a polyethylene glycol lipid (PEG lipid) to the loaded LNP.

21. The method of any one of the preceding claims, wherein the loaded-LNP solution has a pH in a range of from about 4.5 to about 6.0, from about 4.6 to about 5.8, from about 4.8 to about 5.6, from about 5.0 to about 5.5, or from about 5.1 to about 5.4.

22. The method of any one of claims 1-20, wherein the PEG lipid is selected from the group consisting of a PEG-modified phosphatidylethanolamine, a PEG-modified phosphatidic acid, a PEG-modified ceramide, a PEG-modified dialkylamine, a PEG-modified diacylglycerol, and a PEG-modified dialkylglycerol.

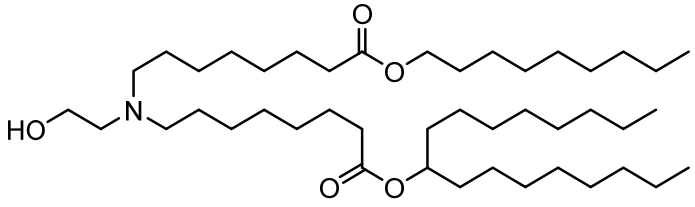
23. The method of any one of claims 1-21, wherein the structural lipid is selected from the group consisting of cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, alpha-tocopherol, and derivatives thereof.

24. The method of any one of claims 1-22, wherein the phospholipid is selected from the group consisting of 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine

(DSPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-*sn*-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-*sn*-glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine,1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin, and derivatives thereof.

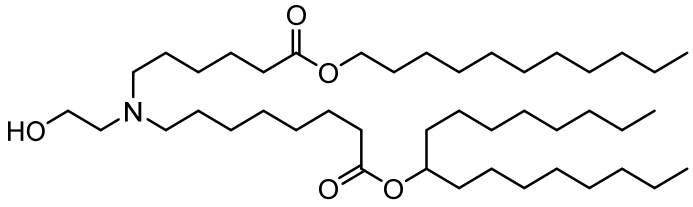
25. The method of any one of claims 1-23, wherein the ionizable lipid comprises an ionizable amino lipid.

26. The method of any one of claims 1-23, wherein the ionizable lipid is



, or a salt thereof.

27. The method of any one of claims 1-23, wherein the ionizable lipid is



, or a salt thereof.

28. An empty LNP prepared by the method of any one of the preceding claims.