can undergo a lyophilization process to provide stable, solid lyophile forms for long-term storage of a nucleic acid therapeutic.

[0013] Embodiments of this invention include the following:

[0014] A composition for making a solid lyophile of lipid nanoparticles comprising one or more nucleic acid active agents, the composition comprising:

an aqueous suspension of the lipid nanoparticles in a pharmaceutically acceptable solution, wherein the lipid nanoparticles encapsulate the one or more nucleic acid active agents;

a dextrin compound; and

a saccharide sugar compound.

[0015] The composition above, wherein the total amount of the dextrin and sugar compounds is from 2% to 20% (w/v) of the composition.

[0016] The composition above, wherein the dextrin compound is from 40 % to 70% (w/v) of the total amount of the dextrin and sugar compounds.

[0017] The composition above, wherein the dextrin compound is from 40 % to 55% (w/v) of the total amount of the dextrin and sugar compounds.

[0018] The composition above, wherein the dextrin compound is 40% to 45% (w/v) of the total amount of the dextrin and sugar compounds.

[0019] The composition above, wherein upon lyophilization and reconstitution of the composition, the change of average size of the nanoparticles is within 10% of their size in the original composition.

[0020] The composition above, wherein upon lyophilization, storage and reconstitution of the composition, the change of average size of the nanoparticles is within 10% of their size in the original composition.

[0021] The composition above, wherein the lyophilized composition is stored at 5°C for at least one month.

[0022] The composition above, wherein the lyophilized composition is stored at -20°C for at least one month.

acceptable solution;

adding a dextrin compound to the solution containing the lipid nanoparticles;

adding a saccharide sugar compound to the solution containing the lipid nanoparticles;

lyophilizing the solution containing the lipid nanoparticles, thereby forming a solid lyophile;

reconstituting the lyophile in a pharmaceutically acceptable carrier, thereby forming a nucleic acid drug product.

[0040] The process above, wherein the total amount of the dextrin and saccharide sugar compounds is from 2% to 20% (w/v) of the solution containing the lipid nanoparticles.

[0041] The process above, wherein the dextrin compound is from 40% to 70% (w/v) of the total amount of the dextrin and saccharide sugar compounds.

[0042] The process above, wherein the dextrin compound is from 40% to 55% (w/v) of the total amount of the dextrin and saccharide sugar compounds.

[0043] The process above, wherein the dextrin compound is 40% to 45% (w/v) of the total amount of the dextrin and saccharide sugar compounds.

[0044] The process above, wherein upon reconstitution, the change of average size of the nanoparticles is within 10% of their size when synthesized.

[0045] The process above, further comprising storing the lyophile before reconstitution.

[0046] The process above, wherein upon storage and reconstitution of the lyophile, the change of average size of the nanoparticles is within 10% of their size when synthesized.

[0047] The process above, wherein the lyophile is stored at 5℃ for at least one month.

[0048] The process above, wherein the lyophile is stored at -20℃ for at least one month.

[0088] In further aspects, the reconstituted suspension can provide stable nanoparticles comparable to that of the suspension before lyophilization. In certain aspects, the average particle size of the nanoparticles can be nearly equal to the size of the nanoparticles in the suspension before lyophilization.

[0089] The compositions and processes of this invention can provide surprising activity and stability of a reconstituted suspension composed of nanoparticles having an encapsulated agent.

[0090] In further aspects, the protected suspension, which can be lyophilized and reconstituted, can contain a protectant composition for lyophilization. A protectant composition of this invention can be composed of a dextrin compound and a saccharide sugar compound. The total amount of the dextrin and sugar compounds may be from 2% to 20% (w/v) of the protected suspension.

[0091] In some embodiments, the dextrin compound can be from 40 % to 70% (w/v) of the total amount of the dextrin and sugar compounds in the protectant composition. In certain embodiments, the dextrin compound can be from 40 % to 55% (w/v) of the total amount of the dextrin and sugar compounds in the protectant composition. In further embodiments, the dextrin compound may be from 40% to 45% (w/v) of the total amount of the dextrin and sugar compounds in the protectant composition. These compositions can provide unexpectedly advantageous properties of a reconstituted nanoparticle suspension, for example, insignificant change of the nanoparticle size or activity.

[0092] In some aspects, upon lyophilization and reconstitution of a protected suspension of nanoparticles, the change of average size of the nanoparticles can be within 10% of their size in the original composition, before lyophilization. In certain aspects, upon lyophilization and reconstitution of a protected suspension of nanoparticles, the change of average size of the nanoparticles can be within 5% of their size in the original composition, before lyophilization.

[0093] This invention contemplates lipid nanoparticle drug formulations containing, for example, siRNA agents, which can be prepared by lyophilization of a suspension of the nanoparticles, and reconstitution of the nanoparticles into a suspension

[00160] Examples of excipients include amino acids such as glycine, alanines, arginine, betaine, leucine, lysine, glutamic acid, aspartic acid, histidine, proline, 4-hydroxyproline, sarcosine, γ-aminobutyric acid, alanopine, octopine, strombine, and trimethylamine N-oxide.

[00161] Examples of excipients include non-ionic surfactants such as polysorbate 20, polysorbate 80, and poloxamer 407.

[00162] Examples of excipients include dispersing agents such as phosphotidyl choline, ethanolamine, acetyltryptophanate, polyethylene glycol, polyvinylpyrrolidone, ethylene glycol, glycerin, glycerol, propylene glycol, sorbitol, xylitol, dextran, and gelatin.

[00163] Examples of excipients include antioxidants such as ascorbic acid, cysteine, thioglycerol, thioglycolic acid, thiosorbitol, and glutathione.

[00164] Examples of excipients include reducing agents such as dithiothreitol, thiols, and thiophenes.

[00165] Examples of excipients include chelating agents such as EDTA, EGTA, glutamic acid, and aspartic acid.

[00166] In some embodiments, the lyophile can be reconstituted using a syringe needle through a stoppered vial. The lyophile can be reconstituted with or without shaking the vial.

[00167] The time for reconstitution can be from 3-30 seconds, or longer.

[00168] In some embodiments, the reconstituted nucleic acid drug product can have less than 0.001% (w/v) of aggregate particles with a size greater than 0.2 μm.

[00169] In certain aspects, the reconstituted nucleic acid drug product can have reduced cytokine activation.

[00170] In additional aspects, the nucleic acid drug product can be reconstituted after a storage time period of six months and retain 80% activity of the nucleic acid agents.

[00171] In some embodiments, the nucleic acid drug product can be reconstituted after a storage time period of six months and the average particle size of the lipid nanoparticles can be less than 125% before lyophilization.

[00172] In certain embodiments, the nucleic acid drug product can be reconstituted after a storage time period of 24 months and retain 90% activity of the nucleic acid agents.

[00173] In further embodiments, the nucleic acid drug product can be reconstituted after a storage time period of 24 months and the average particle size of the lipid nanoparticles can be less than 25% greater than before lyophilization.

[00174] RNAi molecules

[00175] The amount of active RNA interference inducing ingredient formulated in the composition of the present invention may be an amount that does not cause an adverse effect exceeding the benefit of administration. Such an amount may be determined by an in vitro test using cultured cells, or a test in a model animal or mammal such as a mouse, a rat, a dog, or a pig, etc., and such test methods are known to those skilled in the art. The methods of this invention can be applicable to any animal, including humans.

[00176] The amount of active ingredient formulated can vary according to the manner in which the agent or composition is administered. For example, when a plurality of units of the composition is used for one administration, the amount of active ingredient to be formulated in one unit of the composition may be determined by dividing the amount of active ingredient necessary for one administration by said plurality of units.

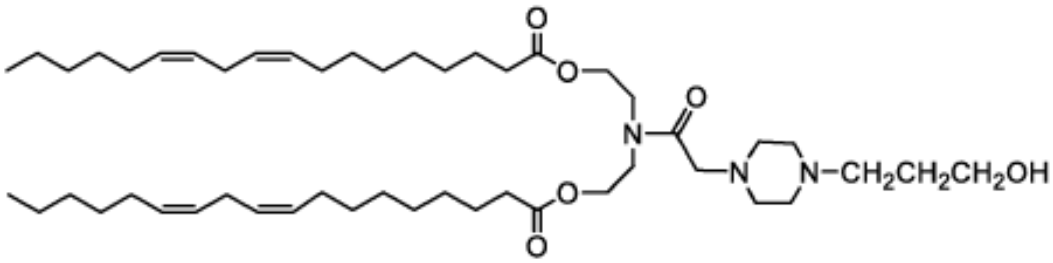
[00177] The nucleic acid molecules and RNAi molecules of this invention can be delivered or administered to a cell, tissue, organ, or subject by direct application of the molecules in liposome formulations to assist, promote or facilitate entry into a cell.

[00178] The nucleic acid molecules and RNAi molecules of this invention can be complexed with cationic lipids, packaged within liposomes, and delivered to target cells or tissues. The nucleic acid or nucleic acid complexes can be locally administered to

which is ((2-(3-(hydroxymethyl)azetidin-1-yl)acetyl)azanediyl)bis(ethane-2,1-diyl) ditetradecanoate.

[00201] Examples of on ionizable lipid include the following compound:

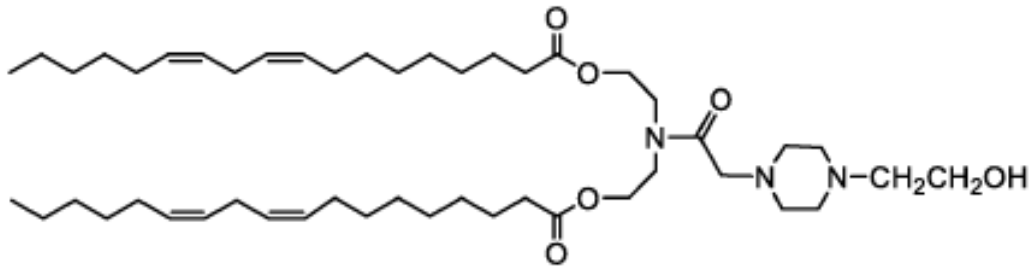
[00202] COMPOUND AA



which is ((2-(4-(2-hydroxyethyl)piperazin-1-yl)acetyl)azanediyl)bis(ethane-2,1-diyl) (9*Z*,9'*Z*,12*Z*,12'*Z*)-bis(octadeca-9,12-dienoate).

[00203] Examples of an ionizable lipid include the following compound:

[00204] COMPOUND AB



which is ((2-(4-(2-hydroxyethyl)piperazin-1-yl)acetyl)azanediyl)bis(ethane-2,1-diyl) (9*Z*,9'*Z*,12*Z*,12'*Z*)-bis(octadeca-9,12-dienoate).

[00205] Examples of on ionizable lipid include the following compound:

[00216] Examples of structural lipids include sterols and zoosterols such as cholesterol, lanosterol, zymosterol, zymostenol, desmosterol, stigmastanol, dihydrolanosterol, and 7-dehydrocholesterol.

[00217] Examples of structural lipids include pegylated cholesterols, and cholestane 3-oxo-(C1-22)acyl compounds, for example, cholesteryl acetate, cholesteryl arachidonate, cholesteryl butyrate, cholesteryl hexanoate, cholesteryl myristate, cholesteryl palmitate, cholesteryl behenate, cholesteryl stearate, cholesteryl caprylate, cholesteryl n-decanoate, cholesteryl dodecanoate, cholesteryl nervonate, cholesteryl pelargonate, cholesteryl n-valerate, cholesteryl oleate, cholesteryl elaidate, cholesteryl erucate, cholesteryl heptanoate, cholesteryl linolelaidate, and cholesteryl linoleate.

[00218] Examples of structural lipids include sterols such as phytosterols, beta-sitosterol, campesterol, ergosterol, brassicasterol, delta-7-stigmasterol, and delta-7-avenasterol.

[00219] Stabilizer lipids

[00220] Examples of stabilizer lipids include zwitterionic lipids.

[00221] Examples of stabilizer lipids include compounds such as phospholipids.

[00222] Examples of phospholipids include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyloleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidylethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoylphosphatidylcholine and dilinoleoylphosphatidylcholine.

[00223] Examples of stabilizer lipids include phosphatidyl ethanolamine compounds and phosphatidyl choline compounds.

[00224] Examples of stabilizer lipids include 1,2-Dioleoyl-*sn*-Glycero-3-Phosphocholine (DOPC).

[00225] Examples of stabilizer lipids include diphytanoyl phosphatidyl ethanolamine (DPhPE) and 1,2-Diphytanoyl-*sn*-Glycero-3-Phosphocholine (DPhPC).

[00325] **Example 7:** Protecting siRNA lipid nanoparticles for long term storage.

[00326] Reconstituted siRNA drug formulations of this invention exhibited long term stability for gene silencing in vivo.

[00327] A siRNA targeted to Hsp47 (GP46) was formulated in liposomal nanoparticles with the following approximate composition: ionizable lipids, 40 mol%, DOPE, 30 mol%, Cholesterol, 25 mol%, and PEG-DMPE, 5.0 mol%.

[00328] The nanoparticle formulations were lyophilized with a protectant composition containing sucrose and (2-hydroxypropyl)-β-cyclodextrin. The total protectant content was from 10% to 12.5%, or from 10% to 15% (w/v). The content of (2-hydroxypropyl)-β-cyclodextrin was 40% (w/v), sucrose 60%. The cyclodextrin was CAVITRON W7 HP5 PHARMA cyclodextrin.

[00329] The vials were stored at temperatures shown in Table 7 for 4 weeks. After storage and reconstitution, the average size of the nanoparticles (PS, Z-avg) was surprisingly stable, as shown in Table 7.

[00330] As shown in Table 7, the size of the siRNA nanoparticles was within 4% of their size in the original composition.

Table 7: Nanoparticle characteristics

Sample total % protectant	Temperature -20C				Temperature 5C			
	Initial Z-avg		1 mo Z-avg		Initial Z-avg		1 mo Z-avg	
	Z-avg	PDI	Z-avg	PDI	Z-avg	PDI	Z-avg	PDI
10	103	0.12	99	0.15	103	0.12	102	0.14
12.5	101	0.13	101	0.15	101	0.13	100	0.16
15	102	0.13	---	---	102	0.13	102	0.15
10	100	0.13	99	0.17	100	0.13	96	0.16
12.5	95	0.16	94	0.14	95	0.16	94	0.14
15	96	0.13	95	0.13	96	0.13	96	0.13
10	95	0.15	95	0.16	95	0.15	96	0.15
12.5	95	0.15	92	0.11	95	0.15	93	0.14
15	94	0.13	92	0.14	94	0.13	91	0.14

[00331] **Example 8:** Protecting siRNA lipid nanoparticles for long term storage.

[00332] Reconstituted siRNA drug formulations of this invention exhibited long term stability for gene silencing in vivo.

[00333] A siRNA targeted to Hsp47 (GP46) was formulated in liposomal nanoparticles with the following approximate composition: ionizable lipids, 40 mol%, DOPE, 30 mol%, Cholesterol, 25 mol%, and PEG-DMPE, 5.0 mol%.

[00334] The nanoparticle formulations were lyophilized with a protectant composition containing sucrose and (2-hydroxypropyl)-β-cyclodextrin. The total protectant content was from 10% to 12.5%, or from 10% to 15% (w/v). The content of (2-hydroxypropyl)-β-cyclodextrin was 40% (w/v), sucrose 60%. The cyclodextrin was CAVITRON W7 HP7 PHARMA cyclodextrin.

[00335] The vials were stored at temperatures shown in Table 8 for 4 weeks. After storage and reconstitution, the average size of the nanoparticles (PS, Z-avg) was surprisingly stable, as shown in Table 8.

[00336] As shown in Table 8, the size of the siRNA nanoparticles was within 5% of their size in the original composition.

Table 8: Nanoparticle characteristics

Sample total % protectant	Temperature -20C				Temperature 5C			
	Initial Z-avg		1 mo Z-avg		Initial Z-avg		1 mo Z-avg	
	Z-avg	PDI	Z-avg	PDI	Z-avg	PDI	Z-avg	PDI
10	103	0.11	99	0.14	103	0.11	99	0.15
12.5	101	0.14	99	0.15	101	0.14	98	0.15
15	102	0.15	---	---	102	0.15	97	0.14
10	96	0.13	95	0.13	96	0.13	93	0.13
12.5	94	0.12	91	0.12	94	0.12	91	0.13
15	94	0.13	89	0.14	94	0.13	91	0.14
10	94	0.15	89	0.15	94	0.15	89	0.13
12.5	91	0.15	87	0.13	91	0.15	88	0.14
15	90	0.14	86	0.15	90	0.14	87	0.12

WHAT IS CLAIMED IS:

1. A composition for making a solid lyophile of lipid nanoparticles comprising one or more nucleic acid active agents, the composition comprising:

an aqueous suspension of the lipid nanoparticles in a pharmaceutically acceptable solution, wherein the lipid nanoparticles encapsulate the one or more nucleic acid active agents;

a dextrin compound; and

a saccharide sugar compound.

2. The composition of claim 1, wherein the total amount of the dextrin and sugar compounds is from 2% to 20% (w/v) of the composition.

3. The composition of claim 1, wherein the dextrin compound is from 40 % to 70% (w/v) of the total amount of the dextrin and sugar compounds.

4. The composition of claim 1, wherein the dextrin compound is from 40 % to 55% (w/v) of the total amount of the dextrin and sugar compounds.

5. The composition of claim 1, wherein the dextrin compound is 40% to 45% (w/v) of the total amount of the dextrin and sugar compounds.

6. The composition of claim 1, wherein upon lyophilization and reconstitution of the composition, the change of average size of the nanoparticles is within 10% of their size in the original composition.

7. The composition of claim 1, wherein upon lyophilization, storage and reconstitution of the composition, the change of average size of the nanoparticles is within 10% of their size in the original composition.

8. The composition of claim 7, wherein the lyophilized composition is stored at 5°C for at least one month.

9. The composition of claim 7, wherein the lyophilized composition is stored at -20°C for at least one month.

10. The composition of claim 1, wherein the nanoparticles have an average diameter of from 45 nm to 110 nm.

11. The composition of claim 1, wherein the concentration of the nucleic acid active agents is from 1 mg/mL to 10 mg/mL.

12. The composition of claim 1, wherein the lipid nanoparticles comprise a compound selected from compound A6, compound A9, compound AA, compound AB, compound C2, compound F5, compound F7, compound C24, and HEDC.

13. The composition of claim 1, wherein the one or more nucleic acid active agents are RNAi molecules capable of mediating RNA interference.

14. The composition of claim 13, wherein the RNAi molecules are siRNAs, shRNAs, ddRNAs, piRNAs, or rasiRNAs.

15. The composition of claim 1, wherein the one or more nucleic acid active agents are miRNAs, antisense RNAs, plasmids, hybrid oligonucleotides, or aptamers.

16. The composition of claim 1, wherein the pharmaceutically acceptable solution is a HEPES buffer, a phosphate buffer, a citrate buffer, or a buffer containing Tris(hydroxymethyl)aminomethane.

17. The composition of claim 1, wherein the dextrin compound is a cyclodextrin.

18. The composition of claim 17, wherein the cyclodextrin compound has one or more of the 2, 3 and 6 hydroxyl positions substituted with sulfoalkyl, benzenesulfoalkyl, acetoalkyl, hydroxyalkyl, hydroxyalkyl succinate, hydroxyalkyl malonate, hydroxyalkyl glutarate, hydroxyalkyl adipate, hydroxyalkyl, hydroxyalkyl maleate, hydroxyalkyl oxalate, hydroxyalkyl fumarate, hydroxyalkyl citrate, hydroxyalkyl tartrate, hydroxyalkyl malate, or hydroxyalkyl citraconate groups.

19. The composition of claim 17, wherein the cyclodextrin compound is (2-hydroxypropyl)-β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin succinate, (2-hydroxypropyl)-γ-cyclodextrin, or 2-hydroxypropyl-γ-cyclodextrin succinate.

36. The process of claim 31, wherein upon reconstitution, the change of average size of the nanoparticles is within 10% of their size when synthesized.

37. The process of claim 31, further comprising storing the lyophile before reconstitution.

38. The process of claim 37, wherein upon storage and reconstitution of the lyophile, the change of average size of the nanoparticles is within 10% of their size when synthesized.

39. The process of claim 37, wherein the lyophile is stored at 5°C for at least one month.

40. The process of claim 37, wherein the lyophile is stored at -20°C for at least one month.

41. The process of claim 31, wherein the nanoparticles have an average diameter of from 45 nm to 110 nm.

42. The process of claim 31, wherein the concentration of the nucleic acid active agents is from 1 mg/mL to 10 mg/mL.

43. The process of claim 31, wherein the one or more nucleic acid active agents are RNAi molecules capable of mediating RNA interference.

44. The process of claim 31, wherein the pharmaceutically acceptable solution is a HEPES buffer, a phosphate buffer, a citrate buffer, or a buffer containing Tris(hydroxymethyl)aminomethane.

45. The process of claim 31, wherein the dextrin compound is a cyclodextrin.

46. The process of claim 45, wherein the cyclodextrin compound has one or more of the 2, 3 and 6 hydroxyl positions substituted with sulfoalkyl, benzenesulfoalkyl, acetoalkyl, hydroxyalkyl, hydroxyalkyl succinate, hydroxyalkyl malonate, hydroxyalkyl glutarate, hydroxyalkyl adipate, hydroxyalkyl, hydroxyalkyl maleate, hydroxyalkyl