

formulations, which lose several of their desired properties including low polydispersity, small particle size, high percentage of encapsulation, and in vivo efficacy upon reconstitution after conventional lyophilization techniques. Thus, new solutions are needed for providing lyophilized lipid nanoparticle compositions that show a maintained integrity and efficacy upon reconstitution.

SUMMARY

[0008] The present disclosure provides lyophilization methods that result in a preservation of lipid nanoparticle integrity, integrity of the encapsulated nucleic acid, the particle size of the lipid nanoparticles within an acceptable degree of pre-lyophilized particle size, and good polydispersity of the nanoparticles. The methods stem from the discovery that specialized excipients can be added to a pretreated suspension of the nanoparticles prior to subjecting the suspension to a lyophilization process. In addition, lyophilization parameters are employed in combination with these excipients to achieve high quality lyophilized lipid nanoparticle product. The lyophilized product is easily reconstituted and readily administered as a pharmaceutical preparation.

[0008a] In an aspect of the present disclosure, there is provided a lyophilized composition comprising lipid nanoparticles encapsulating an RNA, poloxamer, potassium sorbate, and a sugar.

[0008b] In another aspect, there is provided a method of preserving a lyophilized composition as defined above comprising storing the lyophilized product at a temperature of about 2 °C to about 8 °C.

[0008c] In another aspect, there is provided a method of preserving a lyophilized composition as defined above comprising storing the lyophilized product at a temperature of about -20 °C.

[0008d] In another aspect, there is provided a method of reconstituting a lyophilized composition as defined above comprising adding a liquid medium to the lyophilized composition.

[0008e] In another aspect, there is provided a use of a lyophilized composition as defined above reconstituted in a liquid medium in the preparation of a medicament for the treatment of a disease.

[0009] In some examples, a method of lyophilizing a composition comprising lipid nanoparticles encapsulating an RNA is provided comprising the steps of providing a suspension of the lipid nanoparticles in a liquid medium, adjusting the liquid medium

thereby forming a pretreated suspension comprising at least one excipient selected from potassium sorbate, thiosulfate, sodium benzoate, and iodixanol; and subjecting the pretreated suspension to a lyophilization process.

[0010] In another example, a lyophilized composition is provided comprising lipid nanoparticles encapsulating a nucleic acid and one or more excipients selected from potassium sorbate, thiosulfate, sodium benzoate, and iodixanol.

[0011] In another examples, a method of preserving a lyophilized composition of the disclosure is provided comprising storing the lyophilized product at a temperature of about -20 °C to about 8 °C. In some examples, a method of preserving a lyophilized composition of the disclosure is provided comprising storing the lyophilized product at a temperature of about -20 °C to about 25 °C. In some examples, the lyophilized product is stored at about -20 °C. In some examples, the lyophilized product is stored from about 2 °C to about 8 °C. In some examples, the lyophilized product is stored from about 20 °C to about 25 °C.

[0012] In another examples, a method of reconstituting a lyophilized composition of the disclosure is provided comprising adding a liquid medium to the lyophilized composition.

[0013] In another examples, a method of treating a disease or disorder in a subject is provided comprising administering to the subject a lyophilized composition of the disclosure reconstituted in a liquid medium.

[0014] Additional features and advantages of the subject technology will be set forth in the description below, and in part will be apparent from the description, or may be learned by practice of the subject technology. The advantages of the subject technology will be realized and attained by the written description and examples hereof.

[0015] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory and are intended to provide further explanation of the subject technology.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] **Figure 1** shows the characterization (particle size, (PS) polydispersity (PDI), and percent encapsulation (Encap (%)) of reconstituted lipid nanoparticles prepared according to the experiments described in Example 3 for formulations prepared at a concentration of 1 mg RNA/mL.

[0017] **Figure 2A** shows particle size measurements (bar chart) and percent encapsulation (circles) for formulations prepared using P188 poloxamer added to the suspension pre-lyophilization as described in Example 3.

[0018] **Figure 2B** shows particle size measurements (bar chart) and percent encapsulation (circles) for formulations prepared using P188 poloxamer added to the formulation post-lyophilization as described in Example 3.

[0019] **Figure 3** shows the concentration dependence for lipid nanoparticle concentration (0.25, 0.5, and 1.0 mg RNA/mL) for particle size (bar chart) and percent encapsulation (circles) for P188 formulations treated post-lyophilization at different concentrations of P188 as described in Example 3.

[0020] **Figure 4** shows human erythropoietin (hEPO) expression levels for selected reconstituted formulations in comparison to the freeze-thaw control and PBS negative control as described in Example 12.

WHAT IS CLAIMED IS:

1. A lyophilized composition comprising lipid nanoparticles encapsulating an RNA, poloxamer, potassium sorbate, and a sugar.
2. The composition of claim 1, wherein the poloxamer is poloxamer 188.
3. The composition of claim 1 or claim 2, wherein the lyophilized composition comprises about 0.001 to about 1.0 % w/w of the RNA.
4. The composition of claim 3, wherein the lyophilized composition comprises about 0.04 to about 0.2 % w/w of the RNA.
5. The composition of any one of claims 1 to 4, wherein the lyophilized composition comprises from about 0.5 to about 5.0 % w/w of lipids.
6. The composition of any one of claims 1 to 5, wherein the lyophilized composition comprises about 0.5 to about 2.5 % w/w of tris(hydroxymethyl)aminomethane (TRIS) buffer.
7. The composition of any one of claims 1 to 6, wherein the lyophilized composition further comprises from about 0.75 to about 2.75 % w/w of NaCl.
8. The composition of any one of claims 1 to 7, wherein the lyophilized composition comprises from about 85 to about 96 % w/w of the sugar.
9. The composition of any one of claims 1 to 8, wherein the sugar is sucrose.
10. The composition of any one of claims 1 to 9, wherein the lyophilized composition comprises about 0.01 to about 1.0 % w/w of the poloxamer.
11. The composition of any one of claims 1 to 10, wherein the poloxamer is poloxamer 188.
12. The composition of any one of claims 1 to 11, wherein the lyophilized composition comprises from about 0.5 to about 5.0 % w/w of potassium sorbate.
13. A method of preserving a lyophilized composition of any one of claims 1 to 12 comprising storing the lyophilized product at a temperature of about 2 °C to about 8 °C.
14. A method of preserving a lyophilized composition of any one of claims 1 to 12 comprising storing the lyophilized product at a temperature of about -20 °C.
15. A method of reconstituting a lyophilized composition of any one of claims 1 to 12 comprising adding a liquid medium to the lyophilized composition.
16. The method of claim 15, wherein the liquid medium is an aqueous medium.
17. The method of claim 15 or claim 16, wherein the liquid medium further comprises a buffer having a pH of about 7.0 to about 8.5.

18. Use of a lyophilized composition of any one of claims 1 to 12 reconstituted in a liquid medium in the preparation of a medicament for the treatment of a disease.

19. The use of a lyophilized composition of claim 18, wherein the reconstituted lyophilized composition is to be administered intravenously, mucosally, or subcutaneously.

20. The use of a lyophilized composition of claim 18, wherein the reconstituted lyophilized composition is to be administered intramuscularly.

21. The use of a lyophilized composition of claim 18, wherein the reconstituted lyophilized composition is to be administered via inhalation.