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Semple et al.

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(54) **LIPID-ENCAPSULATED POLYANIONIC NUCLEIC ACID**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 169 days.

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(65) **Prior Publication Data**

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Related U.S. Application Data

(63) Continuation of application No. 09/078,954, filed on May 14, 1998, now Pat. No. 6,287,591, which is a continuation-in-part of application No. 08/856,374, filed on May 14, 1997, now abandoned.

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C12N 11/02; C12N 15/88; C07H 21/00

(52) **U.S. Cl.** **424/450**; 428/402.2; 435/177; 435/458; 514/44; 536/22.1

(58) **Field of Search** 424/450; 428/402.2; 435/174, 177; 1/458; 514/44; 536/22.1, 23.1

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(57) **ABSTRACT**

Methods for the preparation of a lipid-nucleic acid composition are provided. According to the methods, a mixture of lipids containing a protonatable or deprotonatable lipid, for example an amino lipid and a lipid such as a PEG- or Polyamide oligomer-modified lipid is combined with a buffered aqueous solution of a charged therapeutic agent, for example polyanionic nucleic acids, to produce particles in which the therapeutic agent is encapsulated in a lipid vesicle. Surface charges on the lipid particles are at least partially neutralized to provide surface-neutralized lipid-encapsulated compositions of the therapeutic agents. The method permits the preparation of compositions with high ratios of therapeutic agent to lipid and with encapsulation efficiencies in excess of 50%.

21 Claims, 17 Drawing Sheets

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What is claimed is:

1. A lipid-encapsulated polyanionic nucleic acid composition comprising lipid-nucleic acid particles, wherein each lipid-nucleic acid particle is formed from a lipid layer surrounding and encapsulating a central region containing a polyanionic nucleic acid, wherein the lipid layer comprises a titratable lipid comprising a protonatable group having a pKa of from 4 to 11 and a modified lipid selected from among lipids which prevent particle aggregation during lipid-nucleic acid particle formation.

2. The composition of claim 1, wherein the titratable lipid is an amino lipid.

3. The composition of claim 1, wherein the titratable lipid is DODAP.

4. The composition of claim 1, wherein the modified lipid is a polyethylene glycol-modified or polyamide oligomer-modified lipid.

5. The composition of claim 4, wherein the modified lipid is a polyethylene glycol-modified ceramide.

6. The composition of claim 5, wherein the modified lipid is PEG-CerC14 or PEG-CerC20.

7. The composition of claim 6, wherein the titratable lipid is an amino lipid.

8. The composition of claim 6, wherein the titratable lipid is DODAP.

9. The composition of claim 1, wherein the polyanionic nucleic acid is an oligonucleotide with a length of from 5 to 50 nucleotides.

10. The composition of claim 9, wherein the titratable lipid is an amino lipid.

11. The composition of claim 9, wherein the titratable lipid is DODAP.

12. The composition of claim 11, wherein the modified lipid is PEG-CerC14 or PEG-CerC20.

13. The composition of claim 9, wherein the polyanionic nucleic acid contains exclusively phosphodiester linkages.

14. The composition of claim 13, wherein the titratable lipid is an amino lipid.

15. The composition of claim 13, wherein the titratable lipid is DODAP.

16. The composition of claim 15, wherein the modified lipid is PEG-CerC14 or PEG-CerC20.

17. The composition of claim 9, wherein the polyanionic nucleic acid contains at least one modified internucleotidic linkage selected from the group consisting of phosphorothioate, phosphorothioate, phosphoroselenate, boranophosphate, methylphosphate and O-alkyl phosphotriester linkages.

18. The composition of claim 1, wherein the lipid-nucleic acid particles are sized to a mean diameter of from about 70 to 200 nm.

19. The composition of claim 18, wherein the lipid-nucleic acid particles are sized to a mean diameter of from about 90 to 130 nm.

20. The composition of claim 1, wherein the protonable group of the titratable lipid has a pKa of from 4 to 7.

21. The composition of claim 1, wherein the weight to weight ratio of the polyanionic nucleic acid to the lipids in the lipid-nucleic acid particle is greater than 0.1.

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