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(54) **METHODS FOR ENCAPSULATING PLASMIDS IN LIPID BILAYERS**

(75) Inventors: **Jeffery J. Wheeler**, Richmond (CA); **Michael Hope**, Vancouver (CA); **Pieter R. Cullis**, Vancouver (CA); **Marcel B. Bally**, Bowen Island (CA)

(73) Assignee: **Inex Pharmaceuticals Corp.**, Burnaby (CA)

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(58) **Field of Search** ..... 264/4.3, 4.6; 424/450; 436/829; 514/44, 851

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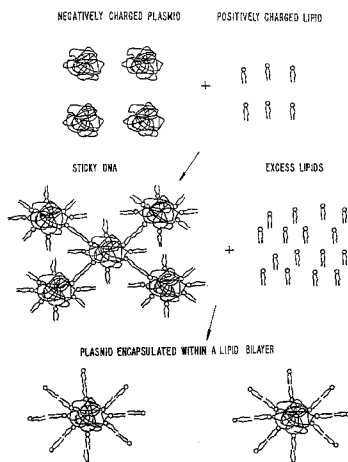
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*Primary Examiner*—Daniel S. Metzmaier  
(74) *Attorney, Agent, or Firm*—Townsend and Townsend and Crew LLP

(57) **ABSTRACT**

Plasmid-lipid particles which are useful for transfection of cells in vitro or in vivo are described. The particles can be formed using either detergent dialysis methods or methods which utilize organic solvents. The particles are typically 65–85 nm, fully encapsulate the plasmid and are serum-stable.

**20 Claims, 18 Drawing Sheets**



What is claimed is:

1. A nucleic acid-lipid particle, said particle comprising a cationic lipid; a non-cationic lipid; a PEG-lipid conjugate; and a nucleic acid.
2. The nucleic acid-lipid particle of claim 1, further comprising a sterol.
3. The nucleic acid-lipid particle of claim 1, wherein said cationic lipid is selected from the group consisting of DODAC, DDAB, DOTAP, DOTMA, DOSPA, DOGS, DC-Chol, and combinations thereof.
4. The nucleic acid-lipid particle of claim 1, wherein said non-cationic lipid is selected from the group consisting of DOPE, POPC, EPC, and combinations thereof.
5. The nucleic acid-lipid particle of claim 1, wherein said cationic lipid comprises from about 2% to about 55% by weight of the total lipid present in said particle.
6. The nucleic acid-lipid particle of claim 1, wherein said cationic lipid comprises from about 5% to about 45% by weight of the total lipid present in said particle.
7. The nucleic acid-lipid particle of claim 1, wherein said cationic lipid comprises from about 5% to about 15% by weight of the total lipid present in said particle.
8. The nucleic acid-lipid particle of claim 1, wherein said cationic lipid comprises from about 40% to about 50% by weight of the total lipid present in said particle.
9. The nucleic acid-lipid particle of claim 1, wherein said non-cationic lipid comprises from about 37% to about 89% by weight of the total lipid present in said particle.
10. The nucleic acid-lipid particle of claim 1, wherein said non-cationic lipid comprises from about 37% to about 75% by weight of the total lipid present in said particle.
11. The nucleic acid-lipid particle of claim 1, wherein said PEG-lipid comprises from about 1% to about 15% by weight of the total lipid present in said particle.
12. The nucleic acid-lipid particle of claim 1, wherein said PEG-lipid comprises about 10% by weight of the total lipid present in said particle.
13. The particle of claim 1, wherein said nucleic acid is DNA.
14. The particle of claim 13, wherein said DNA is a plasmid.
15. The nucleic acid-lipid particle of claim 1, wherein the nucleic acid in said nucleic acid-lipid particle is not substantially degraded after incubation of said serum in serum at 37° C. for 30 minutes.
16. A pharmaceutical composition comprising a nucleic acid-lipid particle comprising a cationic lipid; a non-cationic lipid; a PEG-lipid conjugate; and a nucleic acid; and, a pharmaceutically acceptable carrier.
17. The pharmaceutical composition of claim 16, wherein the nucleic acid-lipid particle further comprises a sterol.
18. The pharmaceutical composition of claim 16, wherein said nucleic acid is DNA.
19. A method of introducing a nucleic acid into a cell, said method comprising contacting said cell with a nucleic acid-lipid particle comprising a cationic lipid, a non-cationic lipid, a PEG-lipid conjugate, and a nucleic acid.
20. The method of claim 19, wherein the nucleic acid-lipid particle further comprises a sterol.

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