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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
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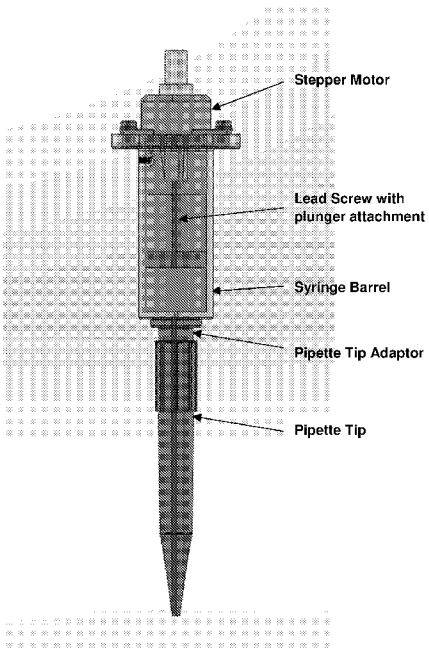
(54) Title: LIPID NANOPARTICLES

FIG. 1



(57) Abstract: The invention features methods and apparatus for producing lipid nanoparticles. Methods of the invention include injecting a lipid solution into an aqueous solution at an automated rate (e.g., a rate controlled by a servo pump). The invention provides methods and apparatus for making lipid nanoparticles possessing a wide range of lipid components and hydrophilic encapsulants, including nucleic acids (e.g., mRNA). Also provided are nanoparticles and compositions thereof made by methods and apparatus of the invention.

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LIPID NANOPARTICLES

BACKGROUND OF THE INVENTION

Nanoparticles are useful for the delivery of various therapeutic, diagnostic, or experimental agents to cells and tissues. Nanoparticles are hypothesized to have enhanced interfacial cellular uptake because of their sub-cellular size, achieving a local effect. It is also hypothesized that there is enhanced cellular uptake of agents encapsulated in nanoparticles compared to the corresponding agent administered in free form. Thus, nanoparticle-entrapped agents have enhanced and sustained concentrations inside cells, thereby increasing therapeutic effects. Furthermore, nanoparticle-entrapped agents are protected from metabolic inactivation before reaching the target site, as often happens upon systemic administration of free agents. Therefore, the effective local nanoparticle dose required for the local pharmacologic effect may be several fold lower than with systemic or oral doses. Lipid nanoparticles, in particular, are useful in enhancing the delivery of agents such as nucleic acids.

Widespread utility of lipid nanoparticles is limited, in part, due to manufacturing and processing constraints. In particular, large-scale production of lipid nanoparticle formulations can introduce variability in lipid nanoparticle characteristics, such as chemical composition, surface charge, size, batch-to-batch concentration, and purity. Such processing limitations have generated a need in the field for new methods and apparatus for synthesizing lipid nanoparticles.

SUMMARY OF THE INVENTION

The invention provides a method for producing lipid nanoparticles, the method including the steps of providing an aqueous solution; providing a lower alkanol solution including lipids; and injecting at an automated rate (e.g., at a rate controlled by a servo pump) the lower alkanol solution to the aqueous solution to produce the lipid nanoparticles. In some embodiments, the steps of the method of the invention are repeated one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) times.

In some embodiments, the lipid nanoparticles have a mean diameter between 80 nm and 100 nm (e.g., between 82 nm and 98 nm, between 84 nm and 96 nm, between 86 nm and 94 nm, between 88 nm and 92 nm, about 80 nm, about 81 nm, about 82 nm, about 83 nm, about 84 nm, about 85 nm, about 86 nm, about 87 nm, about 88 nm, about 89 nm, about 90 nm, about 91 nm, about 92 nm, about 93 nm, about 94 nm, about 95 nm, about 96 nm, about 97 nm, about 98 nm, about 99 nm, or about 100 nm) and a polydispersity index of 0.25 or less (e.g., 0.25, 0.24, 0.23, 0.22, 0.21, 0.20, 0.19, 0.18, 0.17, 0.16, 0.15, 0.14, 0.13, 0.12, 0.11, 0.10, 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02, 0.01, or less).

In some embodiments, the aqueous solution includes a nucleic acid (e.g., DNA, RNA, e.g., mRNA). The nucleic acid may be at a concentration between 50 µg per ml and 200 µg per ml of the aqueous solution (e.g., about 50 µg/ml, about 60 µg/ml, about 70 µg/ml, about 80 µg/ml, about 90 µg/ml, about 100 µg/ml, about 110 µg/ml, about 111 µg/ml, about 111.11 µg/ml, about 120 µg/ml, about 130 µg/ml, about 140 µg/ml, about 150 µg/ml, about 175 µg/ml, or about 200 µg/ml). All of or a portion of the nucleic acid may be encapsulated in the lipid nanoparticles. In some embodiments, the method yields a nucleic acid encapsulation efficiency of at least 80% (e.g., 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater). In some embodiments, the method yields a nucleic acid encapsulation efficiency of at least 94%.

In some embodiments, the lower alkanol solution provides 50% or less (e.g., between 25% and 50%, e.g., 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less) of the total volume. In some embodiments, the lower alkanol solution provides about 33% or about 25% of the total volume.

In some embodiments, the injecting is at a rate from about 1,000 to about 5,000 microliters per second (µl/s; e.g., from about 1,000 to about 5,000 µl/s, from about 1,500 to about 4,500 µl/s, from about 2,000 to about 4,000 µl/s, or from about 2,500 to about 3,000 µl/s). For example, injecting may be at a rate of 2,600 µl/s.

In some embodiments, the aqueous solution further includes a buffer. Examples of suitable buffers include, but are not limited to, a citrate buffer (e.g., 100 mM citrate buffer), a phosphate buffer (e.g., phosphate buffered saline (PBS)), or a TRIS buffer (e.g., TRIS/Sucrose). In some embodiments, the aqueous solution has a pH from about 3.0 to about 8.0 (e.g., about 3.0, about 3.1, about 3.2, about 3.3, about 3.4, about 3.5, about 3.6., about 3.7, about 3.8, about 3.9, about 4.0, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6., about 4.7, about 4.8, about 4.9, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6., about 5.7, about 5.8, about 5.9, about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6., about 6.7, about 6.8, about 6.9, about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, about 7.9, or about 8.0). The aqueous solution can have an osmolality from about 200 mOsm to about 400 mOsm (e.g., from about 250 mOsm to about 350 mOsm, or about 260 mOsm, 270 mOsm, 280 mOsm, 290 mOsm, 300 mOsm, 310 mOsm, 320 mOsm, 330 mOsm, 340 mOsm, or 350 mOsm).

In some embodiments, the lower alkanol solution includes heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate (DLin-MC3-DMA), phosphatidylcholine (1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and polyethylene glycol-dimyristolglycerol (PEG-DMG). The ratio of DLin-MC3-DMA:DSPC:Cholesterol:PEG-DMG may be, for example, 50:10:38.5:1.5.

In some embodiments, the method further includes purifying and/or concentrating the dispersion of lipid nanoparticles, e.g., through the use of a desalting column, dialysis, or tangential flow filtration. Purification and/or concentration may be performed as part of a buffer exchange procedure, e.g., complete buffer exchange. The method may further include sterilizing the dispersion of lipid nanoparticles by filtration, e.g., microfiltration.

In some embodiments, the invention provides a method of producing nanoparticles having an encapsulation efficiency of greater than 90% (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%).

In another aspect, the invention provides a lipid nanoparticle produced by injecting a lower alkanol solution into an aqueous solution having a lipid, wherein the injecting is automated at a rate from about 1,000 to about 5,000 microliters per second (µl/s; e.g., from about 1,000 to about 5,000 µl/s, from about 1,500 to about 4,500 µl/s, from about 2,000 to about 4,000 µl/s, or from about 2,500 to about 3,000 µl/s). In some embodiments, the injecting is at a rate of 2,600 µl/s.

In another aspect, the invention provides an apparatus for producing lipid nanoparticles, the apparatus having an injector configured to transfer a lower alkanol solution from a first reservoir to a

second reservoir configured to hold an aqueous solution; and a servo pump configured to operate the injector at a rate from 1,000 to 5,000 µl/s (e.g., from 1,500 to 4,000 µl/s, 2,000 to 3,500 µl/s, or from 2,500 to 3,000 µl/s. In some embodiments, the servo pump is configured to operate the injector at a rate of 2,600 µl/s.

In some embodiments, the injector is configured to move in three dimensions relative to the second reservoir. In some embodiments, the injector is configured to move in three dimensions relative to the first reservoir and second reservoir.

In some embodiments, the invention provides a pharmaceutical composition including the lipid nanoparticles and a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a schematic diagram of an injector including a stepper motor, lead screw with plunger, syringe barrel, pipette tip adaptor, and pipette tip.

DEFINITIONS

The term “nucleic acid” refers to a molecule of two or more nucleotides or alternative nucleotides. The term, “nucleotide” refers to a nucleoside including a phosphate group. The term “nucleoside” refers to a compound containing a sugar molecule (e.g., a pentose or ribose) or derivative thereof in combination with an organic base (e.g., a purine or pyrimidine) or a derivative thereof (also referred to herein as a “nucleobase”). Examples of nucleic acids include but are not limited to DNA, RNA, tRNA (transfer RNA), mRNA (messenger RNA), siRNA (small interfering RNA), miRNA (micro RNA), shRNA (short hairpin RNA), ncRNA (non-coding RNA), aptamers, ribozymes, and shorter oligonucleotide sequences of any of the foregoing. Alterations of the base, sugar, and phosphate moiety of a nucleotide are encompassed by this definition. Herein, in a nucleotide, nucleoside or polynucleotide (such as the nucleic acids of the invention, e.g., mRNA molecule), the terms “alteration” or, as appropriate, “alternative” refer to alteration with respect to A, G, U or C ribonucleotides. Generally, herein, these terms are not intended to refer to the ribonucleotide alterations in naturally occurring 5′-terminal mRNA cap moieties.

As used herein, the terms “alteration” or “alternative” of a nucleotide, nucleoside, or polynucleotide (such as the polynucleotides of the invention, e.g., mRNA molecule), refer to alteration with respect to A, G, U or C ribonucleotides. Generally, herein, these terms are not intended to refer to the ribonucleotide alterations in naturally occurring 5´-terminal mRNA cap moieties.

As used herein, the term "nanoparticle" refers to a particle having one or a plurality of components, the particle having any one structural feature on a scale of less than about 1000 nm that exhibits novel properties as compared to a bulk sample of the same material or component materials. Routinely, nanoparticles have any one structural feature on a scale of less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 200 nm or less than about 100 nm. In exemplary embodiments, a nanoparticle is a particle having one or more dimensions of the order of about 10-500 nm. In other exemplary embodiments, a nanoparticle is a particle having one or more dimensions of the order of about 10-1000 nm. A spherical nanoparticle would have a diameter, for example, of between 10-100 nm or 10-1000 nm.

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