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(54) **AUTOMATED SYSTEM FOR SCREENING AND OPTIMIZING NANOPARTICLE FORMULATIONS**

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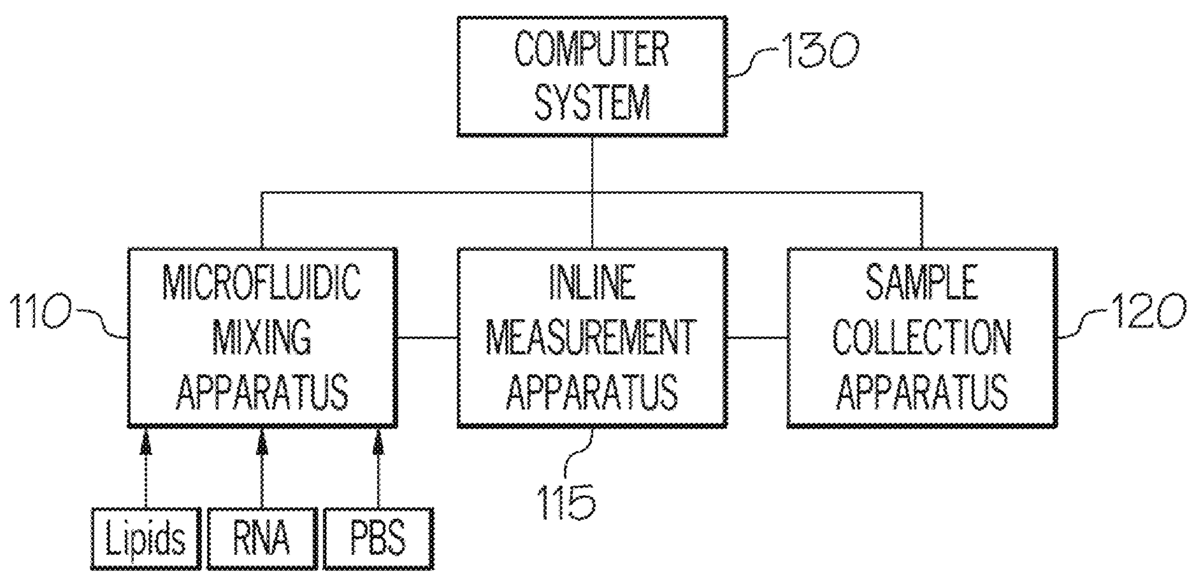
(57) **ABSTRACT**

An apparatus, system, and method comprise receiving, by a computer system, a series of mixing parameters; transmitting, by the computer system, a series of commands corresponding to a series of mixing parameters to a microfluidic mixing system to mix a series of at least two solutions, wherein one of the solutions includes lipids in an organic solvent and the other solution includes ribonucleic acid (RNA) in an aqueous solvent; and generating in response a plurality of formulations of lipid nanoparticles (LNPs) encapsulating the RNA according to the series of mixing parameters.



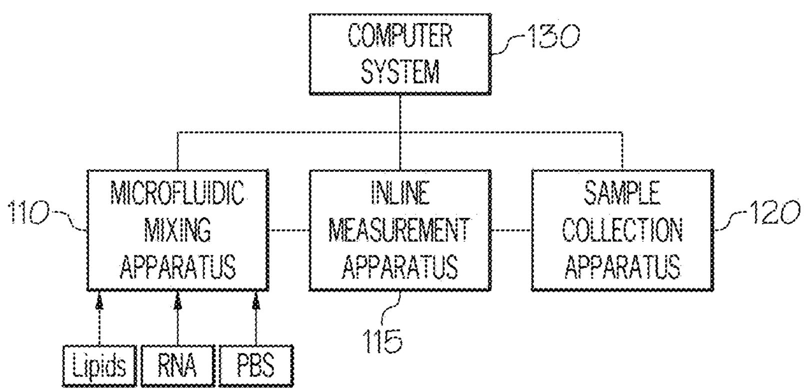

COMPUTER
SYSTEM
130
MICROFLUIDIC
MIXING
APPARATUS
110
INLINE
MEASUREMENT
APPARATUS
115
SAMPLE
COLLECTION
APPARATUS
120
Lipids
RNA
PBS

FIG. 1

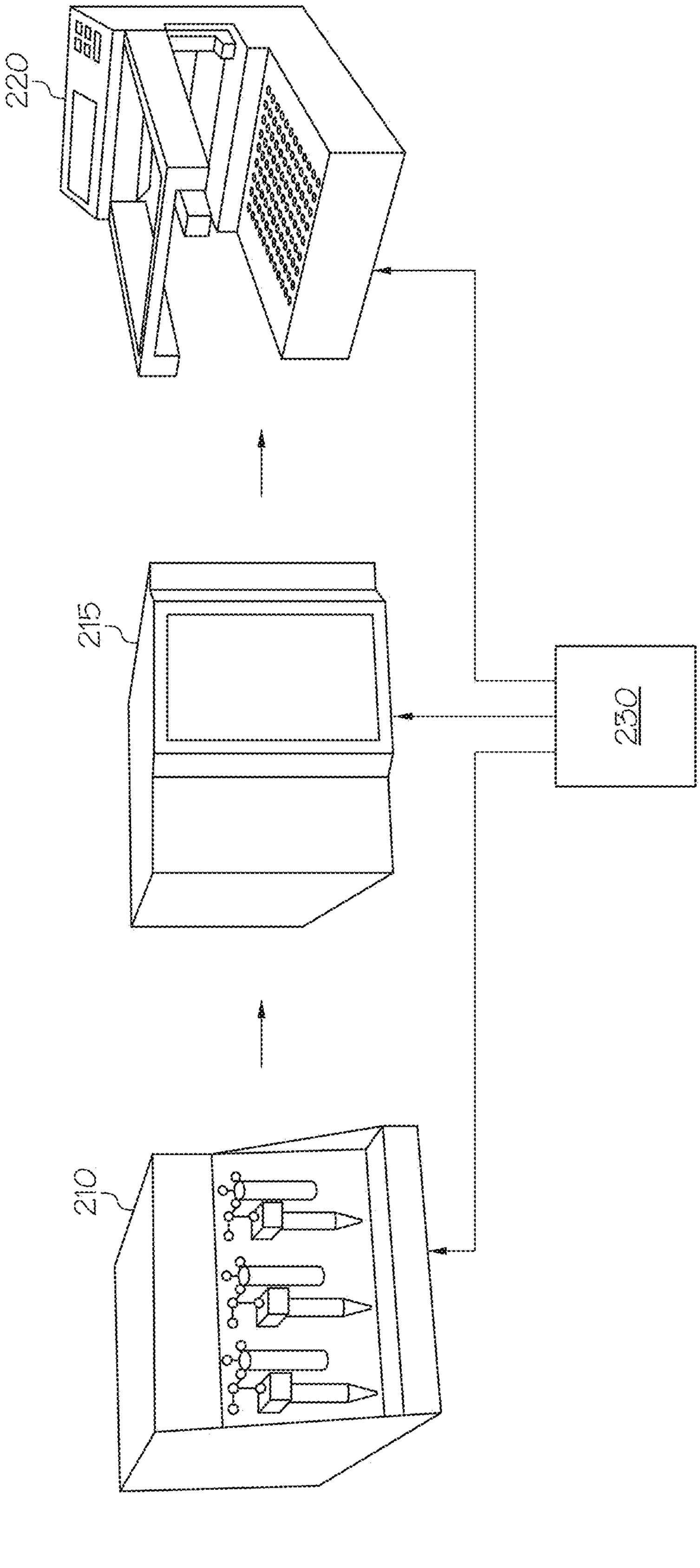


FIG. 2

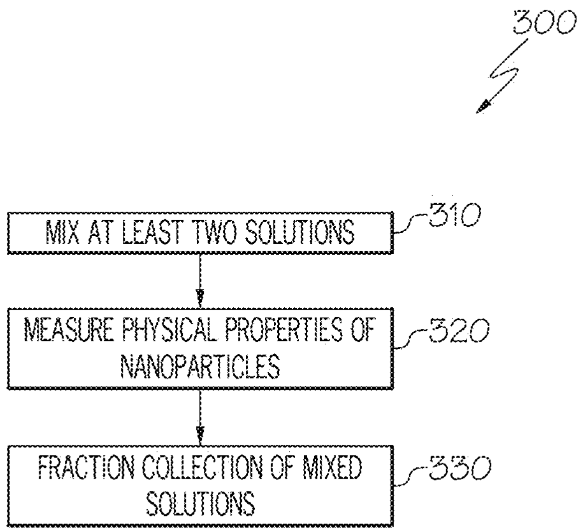

300
MIX AT LEAST TWO SOLUTIONS
310
MEASURE PHYSICAL PROPERTIES OF NANOPARTICLES
320
FRACTION COLLECTION OF MIXED SOLUTIONS
330

FIG. 3

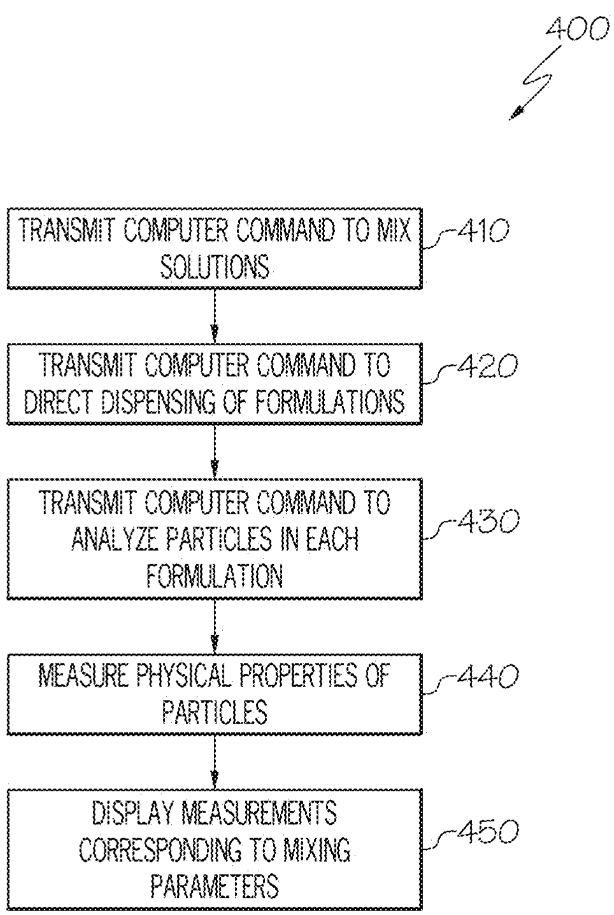

400
TRANSMIT COMPUTER COMMAND TO MIX SOLUTIONS
410
TRANSMIT COMPUTER COMMAND TO DIRECT DISPENSING OF FORMULATIONS
420
TRANSMIT COMPUTER COMMAND TO ANALYZE PARTICLES IN EACH FORMULATION
430
MEASURE PHYSICAL PROPERTIES OF PARTICLES
440
DISPLAY MEASUREMENTS CORRESPONDING TO MIXING PARAMETERS
450

FIG. 4

Start method

1. Accept table consisting of mixing parameters for each sample
   - a) 1. Sample #
   - b) 2. Total sample volume
   - c) 3. Total flow rate (e.g. 10 mL/min)
   - d) 4. Lipid-RNA mixing ratio of (e.g. 1:3)
   - e) 5. Dilution ratio (e.g. 50%)
   - f) 6. Lipid source (e.g. input #1)
   - g) 7. System flush volume
   - h) 8. Measurement delay time

For each sample:

1. Read sample mixing parameters from table
2. Calculate pump operation parameters
   - a) Volume to load each of Pump 1, Pump 2, Pump 3
   - b) Dispense flow rate of each of Pump 1, Pump 2, Pump 3
   - c) (if volume to load any pump is larger than the syringe volume) Number of times to load and dispense in order to achieve the desired total volume
3. Send 'divert to waste' command to sample collector
4. Send 'load and dispense' command to mixing device to dispense flush volume from Pump 3 (diluent pump)
5. Send 'divert to vial' command to sample collector
6. Send 'advance collection vial' command to sample collector
7. Send 'pump load parameters' commands to mixing device to load syringes with the calculated volumes for each pump
8. Send 'start load' command to mixing device
9. Send 'pump dispense parameters' commands to mixing device with the calculated volumes and dispense flow rates for each pump
10. Send 'start dispense' command to mixing device
11. Query mixing device periodically to determine when dispense is complete
12. Repeat load and dispense commands as needed to achieve desired total volume
13. Upon completion of dispensing the desired total volume, wait for the specified measurement delay time, then query inline detector to determine nanoparticle properties
14. Send 'load and dispense' command for system flush volume to mixing device
15. Proceed to next sample

When all samples have been completed

- Prepare and display results table, and save table to file

FIG. 5

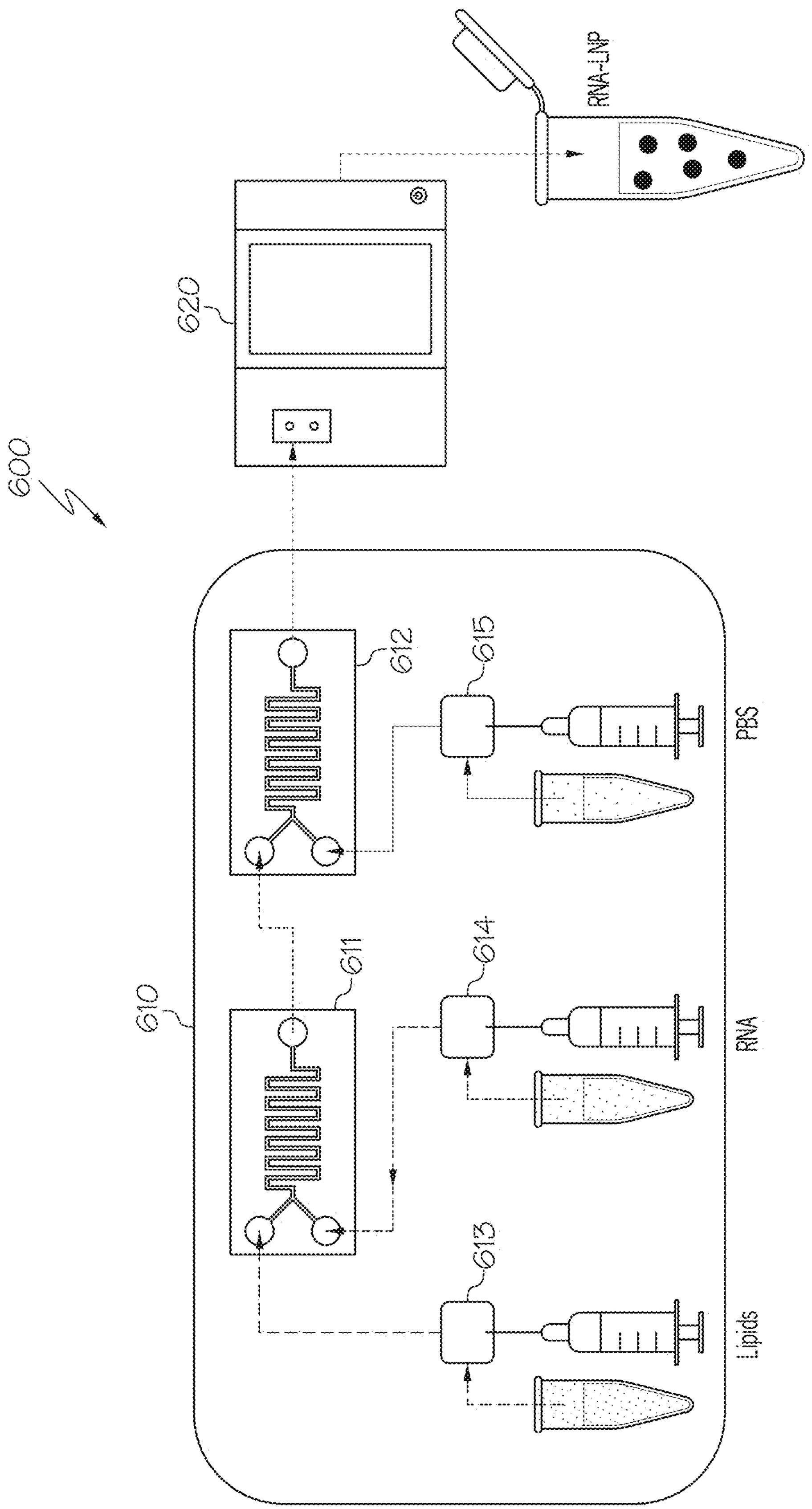
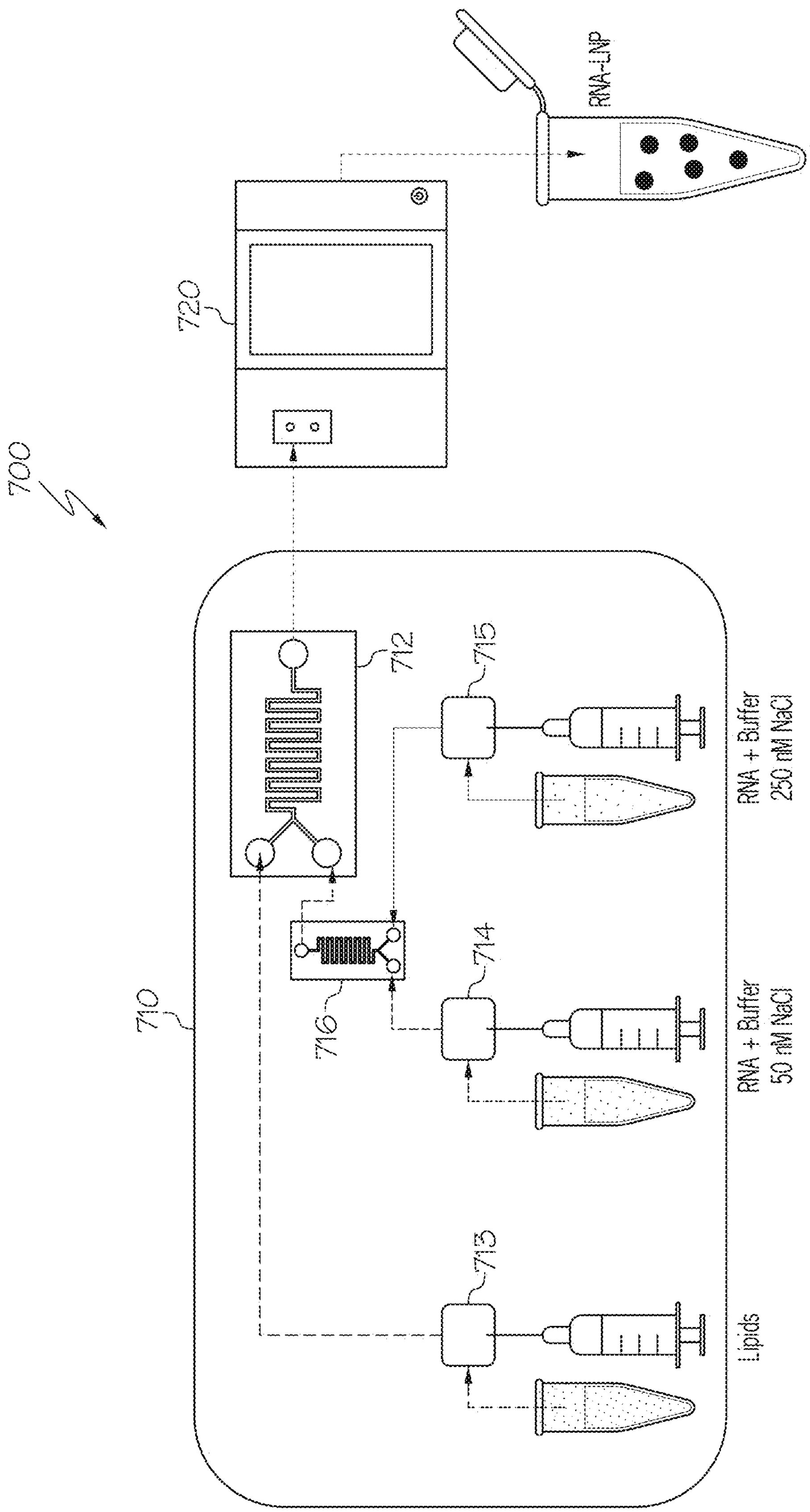


FIG. 6



700
720
RNA-LNP
710
712
716
713
714
715
Lipids
RNA + Buffer
50 nM NaCl
RNA + Buffer
250 nM NaCl

FIG. 7

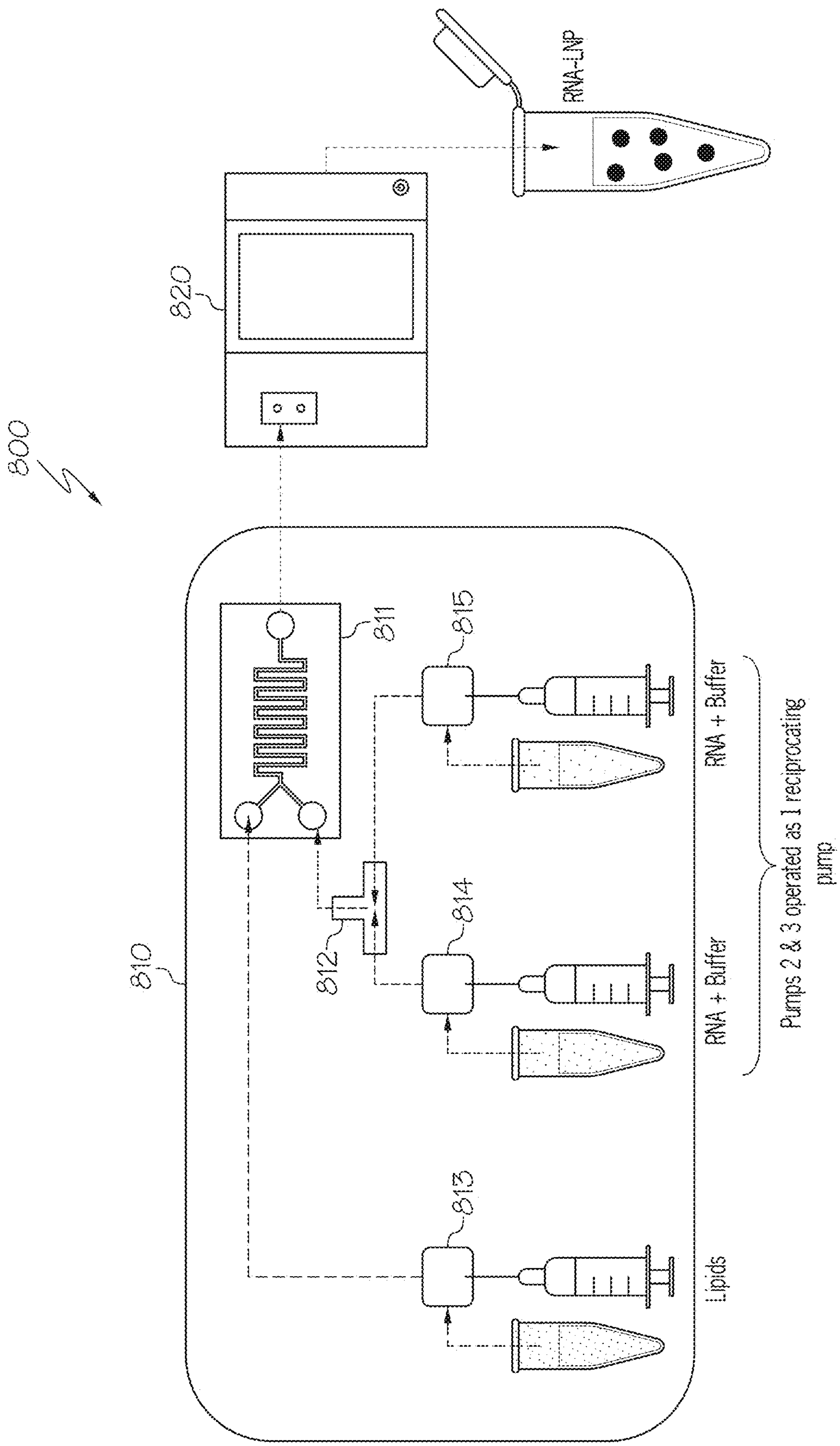

800
810
811
812
813
814
815
820
RNA-LNP
RNA + Buffer
RNA + Buffer
Lipids
Pumps 2 & 3 operated as 1 reciprocating pump

FIG. 8

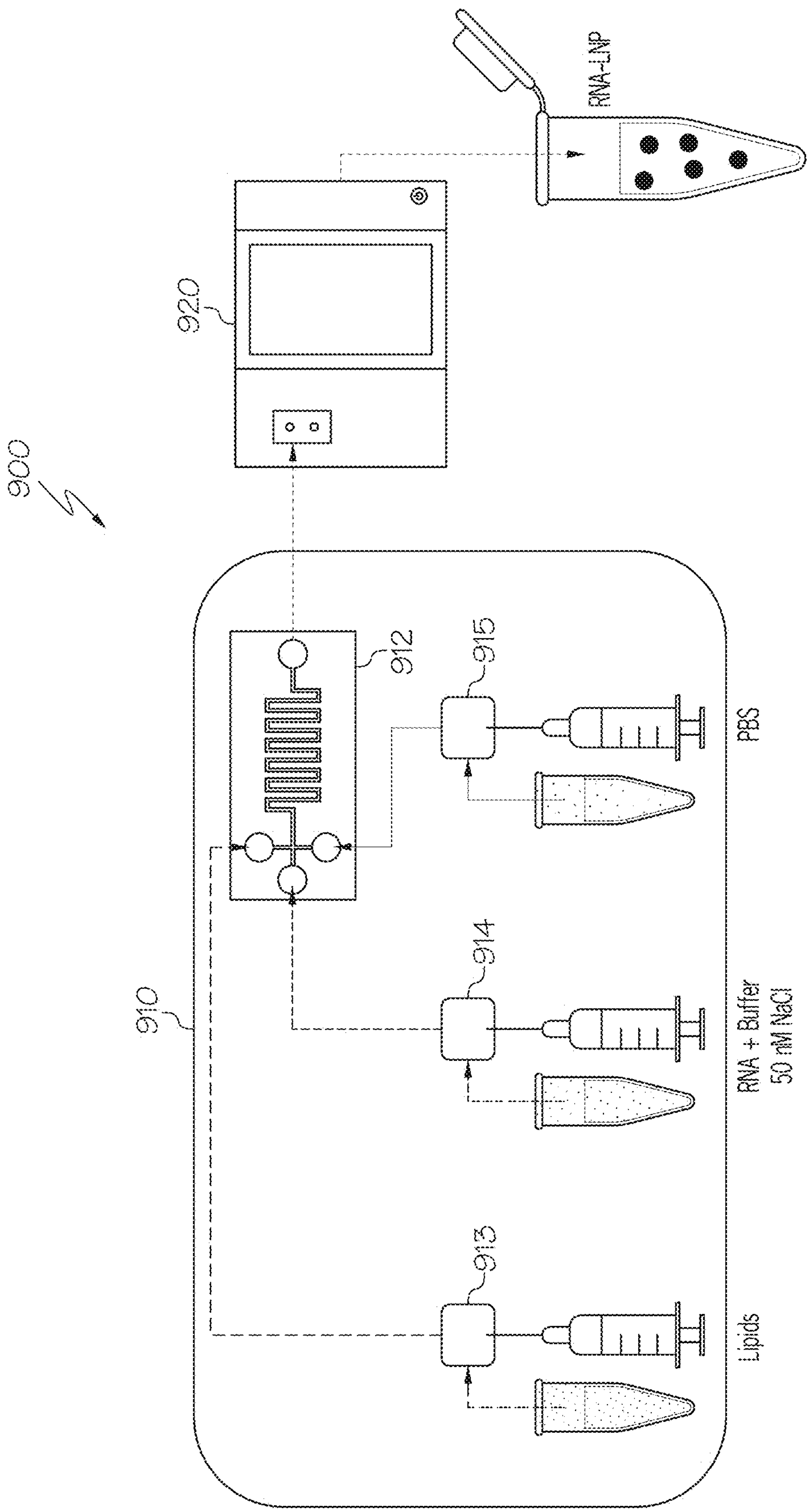
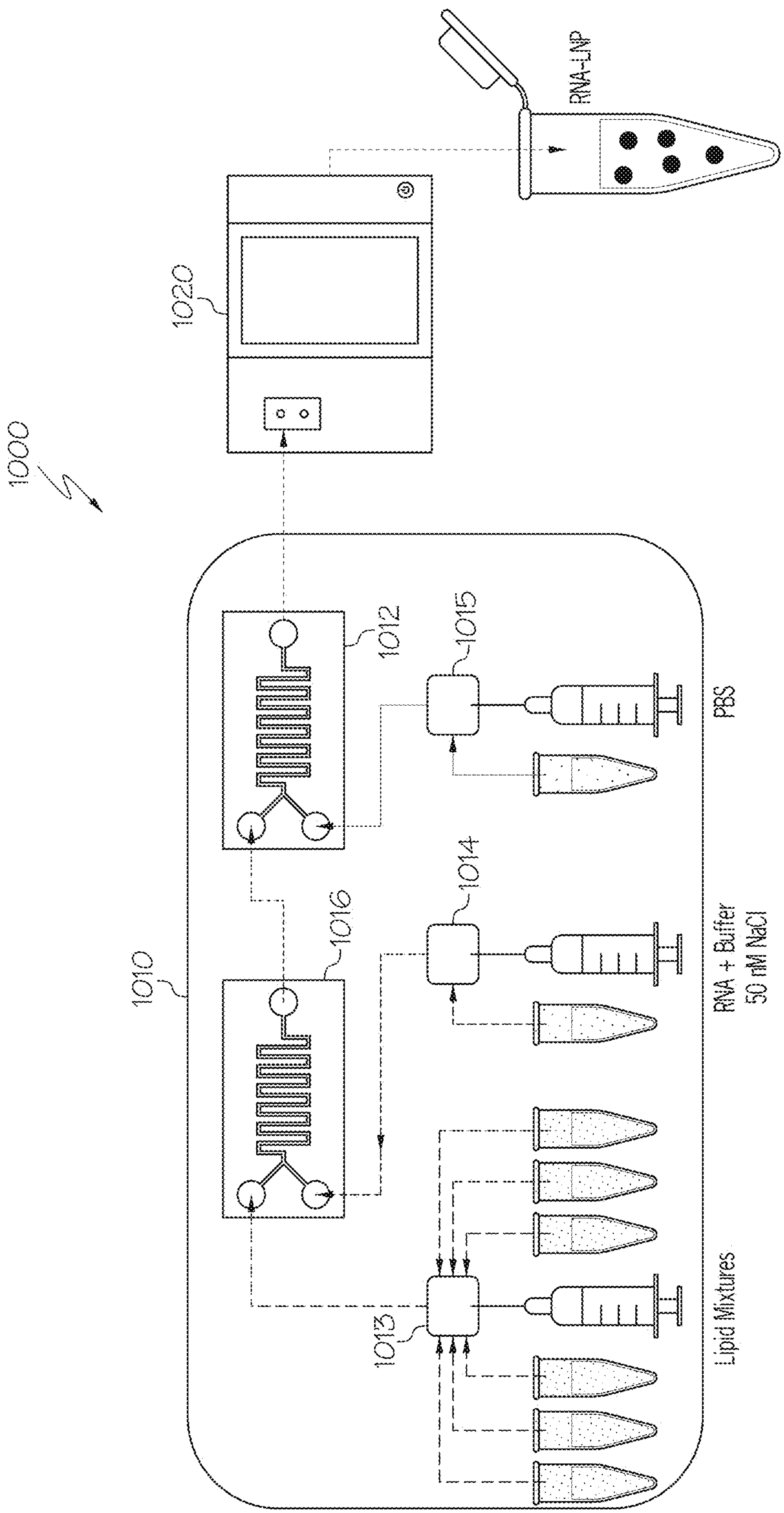

900
910
912
913
914
915
920
RNA-LNP
Lipids
RNA + Buffer
50 nM NaCl
PBS

FIG. 9



1000
1010
1012
1013
1014
1015
1016
1020
RNA-LNP
PBS
RNA + Buffer
50 nM NaCl
Lipid Mixtures

FIG. 10

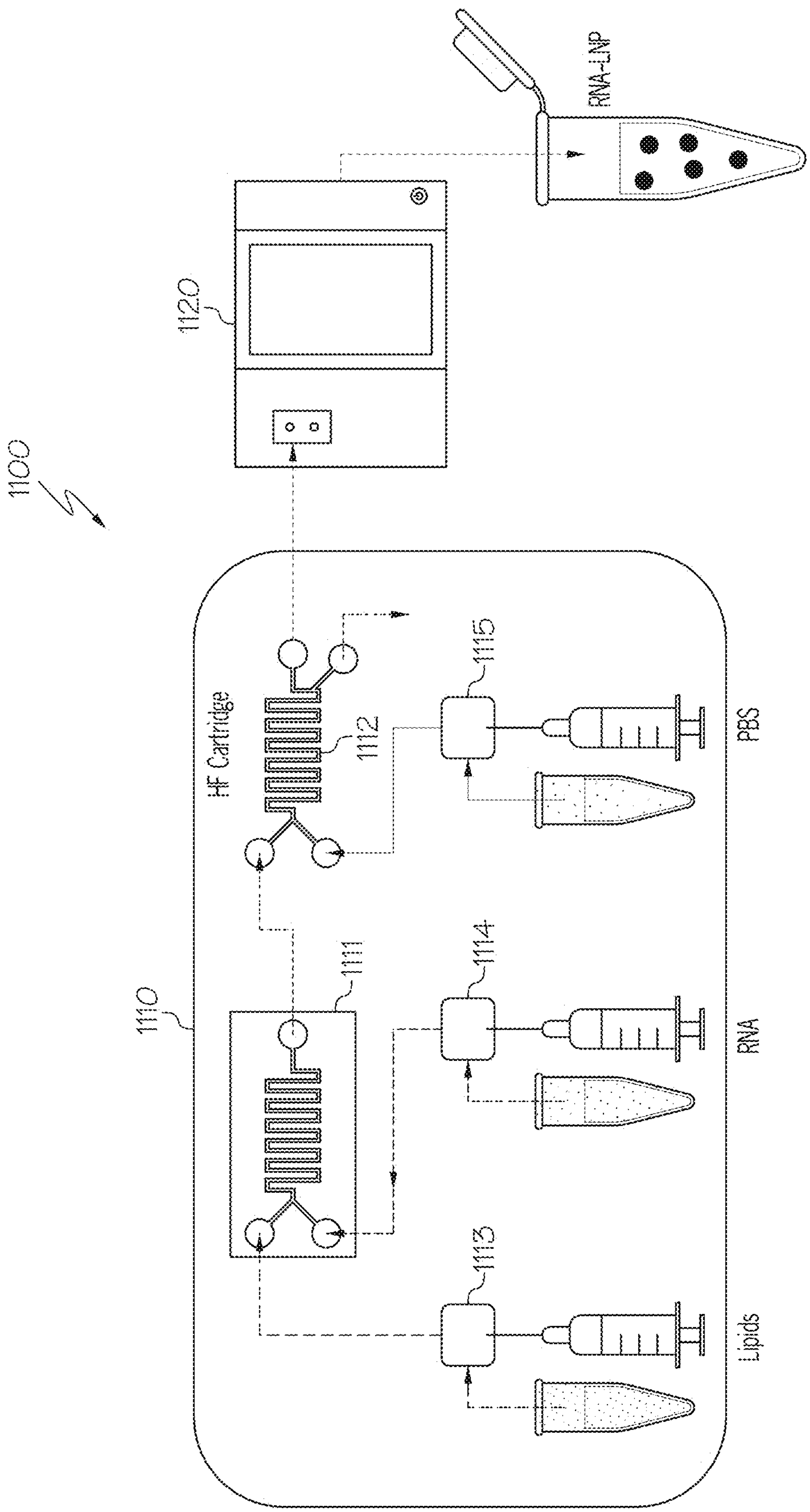

1100
1110
1111
1112
1113
1114
1115
1120
HF Cartridge
Lipids
RNA
PBS
RNA-LNP

FIG. 11

#	Flow rate (mL/min)	Lipid #	Lipid:RNA ratio	[NaCl] (mM)	<R> (nm)	Rh (nm)	PDI	Conc. (1/mL)
1	10	1	1:3	100	50	35	0.1	1.5e10
2	10	1	1:3	150	55	37	0.2	1.4e10
3	15	1	1:3	100	45	32	0.1	1.7e10
4	15	1	1:3	150	50	34	0.18	1.55e10
5	10	1	1:4	100	70	56	0.3	6.0e9
6	10	1	1:4	150	75	58	0.35	4.5e9
7	10	1	1:4	200	76	60	0.35	4.1e9

FIG. 12

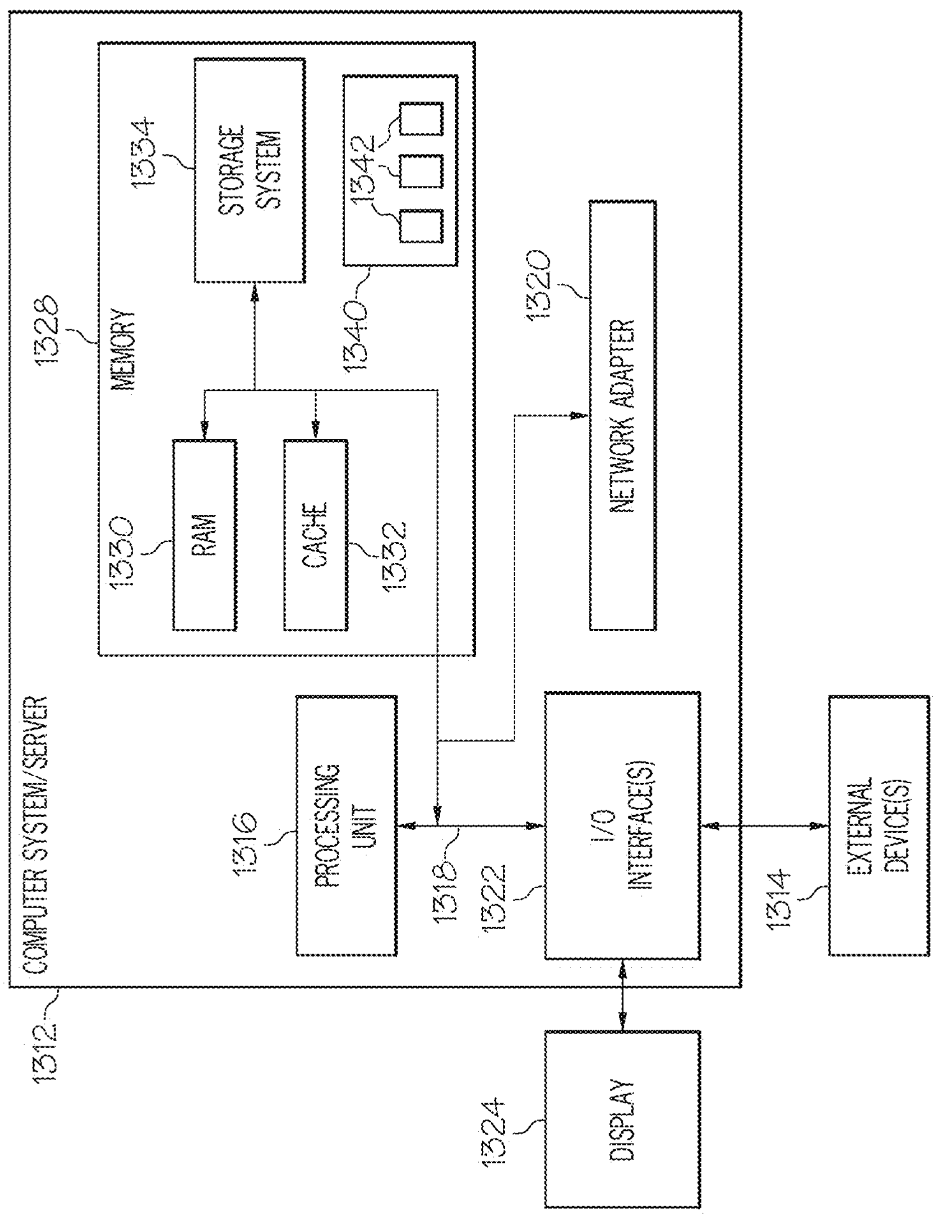

COMPUTER SYSTEM/SERVER
1312
MEMORY
1328
RAM
1330
CACHE
1332
STORAGE SYSTEM
1334
1340
1342
PROCESSING UNIT
1316
1318
NETWORK ADAPTER
1320
I/O INTERFACE(S)
1322
EXTERNAL DEVICE(S)
1314
DISPLAY
1324

FIG. 13

AUTOMATED SYSTEM FOR SCREENING AND OPTIMIZING NANOPARTICLE FORMULATIONS

PRIORITY

[0001] This application claims priority to U.S. provisional patent application No. 63/551,481 filed Feb. 8, 2024 and titled "AUTOMATED SYSTEM FOR SCREENING AND OPTIMIZING NANOPARTICLE FORMULATIONS," the entirety of which is incorporated by reference herein.

BACKGROUND

[0002] The present disclosure relates to adaptive techniques for developing microfluidic formulations, and more specifically, the development of a nanomedicine comprising a carrier nanoparticle encapsulating a molecular payload, such as a ribonucleic acid (RNA) in lipid nanoparticles (LNPs).

[0003] LNP-RNA nanoparticles are expected to become one of the most popular modalities for vaccines and gene therapy. LNP formulations are formulated by microfluidic mixing to encapsulate active pharmaceutical ingredients (APIs). Multiple conditions such as flow rates, solution properties, lipid mixtures, etc. must be tested and screened to produce the optimal LNP-RNA in terms of size, encapsulation efficiency, etc. Similar tests must be run to optimize conditions for formulating other types of nanomedicines that are produced by microfluidic mixing.

[0004] Also, in the course of developing microfluidic formulations of RNA in lipid nanoparticles, many combinations of total flow rate, flow ratio, RNA buffer, and lipid composition must be tested. While instruments are beginning to emerge that automate such screening tasks, they do not incorporate inline analytics for immediate feedback.

SUMMARY

[0005] In one aspect, a computer-implemented method comprises receiving, by a computer system, a series of mixing parameters; transmitting, by the computer system, a series of commands corresponding to a series of mixing parameters to a microfluidic mixing system to mix a series of at least two solutions, wherein one of the solutions includes lipids in an organic solvent and the other solution includes ribonucleic acid (RNA) in an aqueous solvent; and generating in response a plurality of formulations of lipid nanoparticles (LNPs) encapsulating the RNA according to the series of mixing parameters.

[0006] In another aspect, a computer-implemented method comprises receiving, by a computer system, a series of mixing parameters; transmitting, by the computer system, a series of commands corresponding to series of mixing parameters to a microfluidic mixer to mix a series of at least two solutions, wherein one of the solutions includes encapsulating molecules in an organic solvent and the other solution includes payload molecules in an aqueous solvent, resulting in formulations of nano-capsules encapsulating the payload molecules according to the series of mixing parameters; and transmitting, by the computer system, a command to direct the series of formulations to the sample collector such that each formulation is dispensed to a distinct receptacle corresponding to the corresponding mixing parameters.

[0007] In another aspect, an apparatus comprises a microfluidic mixer that mixes a series of at least two solutions, wherein one of the solutions includes encapsulating molecules in an organic solvent and the other solution includes payload molecules in an aqueous solvent; and a sample collector to dispense each formulation to a distinct receptacle corresponding to the corresponding mixing parameters and translate a receptacle to receive dispensed fluid from the mixer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] FIG. **1** depicts a block diagram of a system for processing microfluidic formulations of RNA in lipid nanoparticles at which some embodiments of the present inventive concept can be practiced.

[0009] FIG. **2** depicts a block diagram of a system for processing microfluidic formulations of RNA in lipid nanoparticles at which other embodiments of the present inventive concept can be practiced.

[0010] FIG. **3** depicts a flowchart of a method, in accordance with an embodiment.

[0011] FIG. **4** depicts a flowchart of a method, in accordance with another embodiment.

[0012] FIG. **5** depicts a computer procedure, in accordance with another embodiment.

[0013] FIG. **6** depicts an example of a system configured for condition screening with inline dilution, in accordance with an embodiment.

[0014] FIG. **7** depicts an example of a system configured for condition screening with buffer variation, in accordance with an embodiment.

[0015] FIG. **8** depicts an example of a system comprising a microfluidic mixing system and Multi-Angle Light Scattering (MALS) detection instrument configured for generating large volumes, in accordance with an embodiment.

[0016] FIG. **9** depicts an example of a system configured for condition screening of small volumes, in accordance with an embodiment.

[0017] FIG. **10** depicts an example of a system configured for condition and lipid mixture screening, in accordance with an embodiment.

[0018] FIG. **11** depicts an example of a system configured for condition screening with inline buffer exchange, in accordance with an embodiment.

[0019] FIG. **12** depicts a display output, in accordance with an embodiment.

[0020] FIG. **13** depicts a computer system, in accordance with an exemplary embodiment.

DETAILED DESCRIPTION

[0021] In brief overview, embodiments of the present disclosure describe an apparatus and method that provide a comprehensive solution to fully automated screening integrated with real-time or high-throughput analysis of the resulting nanoparticles. Such a solution may include a fully automated microfluidic mixing system combined with sample collector such as an HPLC fraction collector or the like, and which may optionally integrate with other apparatuses that can, for example, include instruments that measure dynamic and static light scattering (MALS/DLS) in situ in standard microwell plates and/or perform inline analytic operations, but not limited thereto.

[0022] During operation, the mixing system can be programmed by a special-purpose computer to mix up to three, or more, different solutions at preselected conditions in order to produce the specified volume of LNPs. Each sample can be collected by the sample collector into a vial or a well in a well plate. In some embodiments, for example, shown in FIGS. **2** and **3**, a particle analyzer or the like can be positioned between the mixing system and the sample collector for performing in-line analytics, for example, measurements of particle size and concentration during a stop-flow period following a syringe dispense operation. Samples from the sample collector can be tested following a ripening period by a plate reader, or a combination of an autosampler, pump, and particle analysis system for post-formulation analytics.

[0023] Accordingly, embodiments of the present inventive concept include a fully automated microfluidic mixing system capable of formulation screening of many conditions with no manual intervention, combined with inline analytics (MALS/DLS) and a chromatography fraction collector to collectively provide a versatile workflow for screening LNP-RNA formulations resulting in high productivity as compared to conventional manual-intensive systems. This is beneficial for accommodating the rising popularity of LNP-RNA nanoparticles as modalities for vaccines and gene therapy, as well as other nanomedicines produced by similar mixing processes. Current formulation screening systems, on the other hand, require significant manual labor, for example, exchanging vials, filling and replacing syringes, offline analysis and so on, resulting in low productivity.

Definitions

Particle

[0024] A particle may be a constituent of a liquid sample aliquot. Such particles may be molecules of varying types and sizes, nanoparticles, virus like particles, liposomes, emulsions, bacteria, and colloids. These particles may range in size from sub-nanometer to microns.

Analysis of Macromolecular or Particle Species in Solution

[0025] The analysis of macromolecular or particle species in solution may be achieved by preparing a sample in an appropriate solvent and then injecting an aliquot thereof into an analytical system. In some cases, the analysis may take place "in batch", i.e. without separation. In other cases, the analytical system may comprise a sample separation system such as a liquid chromatography (LC) column or field flow fractionation (FFF) channel where the different species of particles contained within the sample are separated into their various constituencies. Once separated, generally based on size, mass, or column affinity, the samples may be subjected to analysis. Analysis in a batch or separation method may be accomplished by measuring light scattering, refractive index, ultraviolet absorption, electrophoretic mobility, and/or viscometrical responses.

[0026] Light scattering (LS) represents a family of non-invasive techniques for characterizing macromolecules and sub-micron particles in solution or suspension. The types of light scattering detection frequently used for the characterization of nanoparticles such as nanomedicines, or more specifically, nanocapsules, are multi-angle static light scattering (MALS), dynamic light scattering (DLS) and electrophoretic light scattering (ELS).

[0027] MALS is a LS technique used to determine molar mass, particle size and particle concentration. MALS involves illumination, by means of a laser beam, of a volume containing solution or suspension of the nanoparticles to be characterized. Light scattered by the nanoparticles is detected simultaneously by multiple photodetectors, each positioned at a different scattering angle with respect to the laser beam. As is known to those skilled in the art, the signals representing the time-averaged scattering intensity as a function of angle are analyzed to determine physio-chemical properties such as molar mass, particle size and particle concentration. Different models may be applied to the angle-dependent intensities so as to interpret the results in terms of spherical radius, rod length, random coil radius and root-mean-square radius.

[0028] DLS, also known as quasi-elastic light scattering (QELS) and photon correlation spectroscopy (PCS), is an LS technique used to determine particle hydrodynamic size, polydispersity and—in combination with static light scattering—particle concentration. Similar to MALS, a volume containing a solution or suspension of macromolecules or nanoparticles is illuminated by means of a laser beam. In a DLS measurement, time-dependent fluctuations in the scattered light intensity are measured using a fast photodetector, typically positioned at a single scattering angle with respect to the laser beam, though methods of utilizing multiple scattering angles are known. As is known to those skilled in the art, DLS measurements determine the diffusion coefficient of the molecules or particles, which can in turn be used to calculate their hydrodynamic radius and polydispersity. DLS measurements may be combined with static light scattering measurements to determine particle concentration and, in the case of multi-modal samples (i.e. samples containing at least two population of sufficiently distinct size), the particle concentrations of each of more than one population.

Electrophoretic Light Scattering

[0029] Electrophoretic light scattering (ELS) is a LS technique used to measure the electrophoretic mobility and zeta potential of nanoparticles in solution or suspension. A laser beam is split into two, with one beam (sample beam) routed through a cell containing the sample to be measured and the other beam (reference beam) routed around the cell to the photodetector. A portion of the light scattered by the sample arrives at a photodetector where it interferes coherently with the reference beam.

[0030] An ELS measurement takes place in a cell containing two electrodes. An electrical field is applied to the electrodes, and particles or molecules that have a net charge, or more strictly a net zeta potential, will migrate towards the oppositely charged electrode with a velocity. As is known to those skilled in the art, the particle velocity and direction of motion are determined by applying a periodic phase shift to the reference beam and measuring the frequency shift of the light scattered by the sample. The ratio of this velocity to the applied electric field is the electrophoretic mobility, which can be expressed as a zeta potential by applying theoretical models. The direction of motion relative to the direction of the applied field is used to determine the sign of the electrophoretic mobility, i.e. positive or negative, which corresponds to the polarity of the charge on the particle.

Overview

[0031] It is well-known that nanoparticles can be produced by microfluidics in a reproducible and accurate manner. The manufacturing technique can be chosen according to the physical properties of the particle used to encapsulate and the encapsulated agent. However, there is a need to provide a comprehensive system to fully automate screening tasks in the course of developing microfluidic formulations including the particle used to encapsulate, i.e., carrier nanoparticle and the agent, i.e., payload, or more specifically, nanocapsules comprising a protective shell with a core comprising mRNA or other bioactive compound.

FIGURES

[0032] FIG. **1** depicts a block diagram of a system **10** at which embodiments of the present inventive concept can be practiced. The system **10** is constructed and arranged to process microfluidic formulations of RNA in lipid nanoparticles, and in doing so may include a microfluidic mixing apparatus **110**, an inline measurement apparatus **115**, a sample collection apparatus **120**, and a special-purpose computer **130** for controlling the mixing operations performed by the microfluidic mixing apparatus **110** and the collection operations of the sample collection apparatus **120**.

[0033] In some embodiments, the mixing apparatus **110** is constructed and arranged to operate on two or more fluid volumes for performing chemical or biochemical reactions to achieve high and repeatable yields. In some embodiments, the mixing apparatus **110** comprises a first input port for receiving a first solution comprising lipids or other particle-forming molecules dissolved in an organic solvent and a second input port for receiving a second solution mRNA or other oligonucleotides or drug molecules in an aqueous solvent. A microfluidic mixing operation is performed on the two solutions (e.g., shown in FIGS. **6-11**) whereby the lipids precipitate and form nanoparticles that encapsulate the mNRA. In some embodiments, the mixing apparatus **110** comprises a third input port for receiving a source of a diluent or buffer solution such as phosphate buffered saline (PBS), which can be mixed with the aqueous and organic solutions input by the first and second input ports, respectively. Accordingly, the mixing apparatus **110** can mix in some embodiments two solutions and in other embodiments three solutions. In other embodiments, the microfluidic mixing apparatus **110** includes at least one computer processor programmed to receive command instructions from the special-purpose computer **130** to mix the solutions provided by the input ports at selected conditions in order to produce the specified volume of LNPs. The mixing operation may result in electrostatic and hydrophobic/hydrophilic interactions that drive the coalescence of nanoparticles, or polymeric particles in the nanometer size range, which can be measured, analyzed, or otherwise processed by other instruments downstream from the mixer **110** as described below. Examples are provided in FIGS. **6-11** and the Appendix attached herewith. Also, shown and described with reference to FIGS. **6-11** are various embodiments of a microfluidic mixing system, which can be similar to or the same as a mixing apparatus of FIGS. **1-3**. For example, in some embodiments, the microfluidic mixing apparatus **110** may be part of a system comprising at least two microfluidic pumps, such as syringe pumps that receive the contents of the inputs for filling and dispensing to provide a more accurate mixing ratios, or a continuous pump drawing from a reservoir, as well as at least one microfluidic mixer, e.g., including at least one microfluidic mixing chip, T-junction mixer, Y-junction mixer, and augmented T-junction mixer, but not limited thereto. The syringe pumps are constructed and arranged to provide accurate mixing ratios, and can be filled with and dispense one volume of solution at a time. In other embodiments, the pumps include a continuous pump drawing solution from a reservoir or the like. The continuous pumps can be selected from the group consisting of peristaltic pumps, pressure-driven pumps, piston pumps, and diaphragm pumps. The input ports can communicate with three computer-controlled syringe pumps together with associated degassers, filters, mixers and valves, such that the syringe pumps can provide a delivery to the sample collection apparatus **120** of a solution at a desired mixing ratio.

[0034] In the course of developing microfluidic formulations of nanoparticles encapsulating mRNA or the like, e.g., or other oligonucleotides or drug molecules, many combinations of total flow rate, flow ratio, RNA buffer and lipid composition may be tested. Accordingly, the mixing apparatus **110** can not only automate such screening tasks, but also integrate with the inline measurement apparatus **115** and sample collection apparatus **120** for generating an output for analytics, and in particular, provide a collective comprehensive solution to fully automate screening integrated with real-time or high-throughput analysis of the resulting nanoparticles. The inline measurement apparatus **115** between the mixing apparatus **110** and the sample collection apparatus **120** allows for immediate feedback on the success of forming nanoparticles of a desired size and/or other characteristics using the specific mixing conditions provided by the computer **130**.

[0035] In some embodiments, as shown in FIG. **1**, the sample collection apparatus **120** is constructed of receiving adapter racks and related collection vessels arranged to collect each sample output from the mixing apparatus **110**, and direct the formulations to a distinct receptacle, e.g., a vial, well in a well plate, or other temporary storage vehicle according to a set of mixing parameters (described below). Said sample outputs consist of formulations of lipid nanoparticles (LNPs) encapsulating the RNA (LNP-RNA—LNP encapsulating RNA). In some embodiments, the sample collection apparatus **120** includes a chromatography fraction collector or the like, which may support racks, microplates, vials, tubes, or other collection vessels to allow samples to be collected in small, fixed volumes, or controlled by the computer **130** to capture the eluting fluid in tubes, vials, or the like. For example, a tray of tubes or plate containing samples may be manually or automatically loaded into the sample collection apparatus **120**, which is controlled by the computer **130** to dispense each sample to a different tube or well. In some embodiments, after the abovementioned mixing, measuring, and collecting steps, samples from the sample collection apparatus **120** can optionally be tested using an autosampler, pump, plate reader, or other analysis device(s). For example, a light scattering measurement instrument (not shown) can be at the output of the sample collection apparatus **120** configured as a sample collector for forming automated measurements on the output of the sample collection apparatus **120**. Samples from the sample collector can be tested following a ripening period by vials or the like by the light scattering measurement instrument using an autosampler and pump, but not limited thereto.

[0036] The special-purpose computer **130** stores and executes algorithms for controlling the microfluidic mixing system **110** and sample collection apparatus **120**, for example, according to processes described in embodiments herein. For example, the computer **130** can control the pumps, injection devices, and processors of the mixing apparatus shown in FIGS. **6-11**. The special-purpose computer **130** is configured to transmit a series of commands corresponding to a series of mixing parameters to the microfluidic mixing system **110** to mix a series of at least two solutions. The computer **130** may receive a series of predetermined mixing parameters, e.g., flow rate, total volume and relative amount of each solution programmed by a user or other computer into the computer **130**, and generate a series of commands corresponding to a series of mixing parameters to the microfluidic mixing system **110** to mix a series of at least two solutions, where one solution includes lipids in an organic solvent and the other solution includes RNA in an aqueous solvent, resulting in formulations of lipid nanoparticles (LNPs) encapsulating the RNA according to the series of mixing parameters. In other embodiments, one of the solutions includes encapsulating molecules in an organic solvent and the other solution includes payload molecules in an aqueous solvent, resulting in formulations of nano-capsules encapsulating the payload molecules (e.g., LNP-RNA—LNP encapsulating RNA) according to the series of mixing parameters. In some embodiments, the computer **130** generates a command to direct the series of formulations to the sample collector **120** such that each formulation is dispensed to a distinct receptacle (vial/well in well plate) corresponding to the corresponding mixing parameters (translate a receptacle to receive dispensed fluid from the mixer **110**). The computer **130** may include a database, or communicate with a database, to store measurements corresponding to the mixing parameters. The computer **130** may include a display, or communicate with a remote display, to display measurements corresponding to the mixing parameters, for example, shown in FIG. **12**.

[0037] FIG. **2** depicts a block diagram of a system **20** for processing microfluidic formulations of RNA in lipid nanoparticles at which other embodiments of the present inventive concept can be practiced. The system **20** is constructed and arranged to process microfluidic formulations of RNA in lipid nanoparticles, and in doing so may include a microfluidic mixing apparatus **210**, a light scattering instrument **215**, a sample collection apparatus **220**, and a special-purpose computer **230**, which may be similar to those counterpart components described above with respect to FIG. **1**. The microfluidic mixing apparatus **210** can, for example, consist of a Calypso™ composition-gradient system by Wyatt Technology that includes three computer-controlled syringe pumps with associated valves, sensors and microfluidic connections.

[0038] In some embodiments, the system **20** also includes an inline measurement apparatus, namely, a light scattering instrument **215** positioned between the microfluidic mixing apparatus **210** and the sample collection apparatus **220** for inline measurement and characterization of nanoparticles during a stop-flow period, following a mixing operation. The light scattering instrument **215** may be used to perform light scattering analysis or the like for the inline measurement of particles. In some embodiments, the light scattering instrument **215** can include a multiangle light scattering (MALS) instrument, for example, an ultraDAWN™ instrument by Wyatt Technology that is normally used to measure a molar mass, size and particle concentration in real-time in order to monitor product attributes inline or online with downstream purification and fill-finish processes. In other embodiments, the light scattering instrument **215** can include a DynaPro™ ZetaStar™ light scattering instrument by Wyatt Technology, which combines simultaneous DLS and electrophoretic mobility measurements to determine size, polydispersity, and zeta potential.

[0039] In some embodiments, the system **20** includes a DLS plate reader (not shown) such as a DynaPro™ Plate Reader for performing a post-formulation analytics operations on the output of the sample collection apparatus.

[0040] The special-purpose computer **230** includes one or more processors, data storage, and peripherals such as input/output devices to communicate electronically with the mixing apparatus **210**, MALS instrument **215**, sample collection apparatus **220**, and/or DLS plate reader **225**. Data exchanges may include the computer **230** generating commands for the mixing apparatus **210**, sample collection apparatus **220**, and light scattering instrument **215**. In some embodiments, the computer **230** generates a series of commands corresponding to a series of mixing parameters to the microfluidic mixing apparatus **210** to mix a series of at least two solutions. In some embodiments, the computer **230** generates at least one command to direct the series of formulations to the sample collector **220** such that each formulation is dispensed to a distinct receptacle (vial/well in well plate) corresponding to the corresponding mixing parameters (translate a receptacle to receive dispensed fluid from the mixer). In some embodiments, the computer **230** generates at least one command to the particle analyzer **215** to analyze LNP-RNA particles in each formulation, resulting in measurements of physical properties of the LNP-RNA particles (e.g., particle size, concentration). In some embodiments, as shown in FIG. **5**, the computer **230** generates at least one command to an inline measurement apparatus **215** for in-line analytics, namely, to analyze nano-capsules in each formulation, resulting in measurements of physical properties of the nano-capsules (e.g., particle size, concentration, etc.). The computer **230** can generate commands, etc. similar to the computer **130** of FIG. **1**, e.g., for controlling the mixer and sample collector.

[0041] During operation, the solutions, e.g., aqueous and organic streams and optionally a buffered saline solution or the like, mixed by the mixer **210** and the resulting mixture collected by the sample collector **220** can be optionally provided to another analysis system such as a DLS plate reader (not shown), which can select wells of a plate of interest from the sample collector **220** and determine one or more properties of a plateful of candidates and formulations. Particle properties, for example, size measurements taken by the particle analyzer **215**, can be periodically measured by the DLS plate reader.

[0042] FIG. **3** depicts a flowchart of a method **300**, in accordance with an embodiment. In describing the method **300**, reference is made to components of a system of FIGS. **1-3**. In some embodiments, the steps in the method **300** are performed by the special-purpose computer **130** of FIG. **1** or **230** of FIG. **2**.

[0043] At block **310**, at least two solutions are mixed together by a microfluidic mixing apparatus. For example, LNPs encapsulating RNA (LNP-RNA) can be formed by mixing a payload and lipid packets comprised of cationic and neutral lipids in respective solutions, one of the solutions may include lipids in an organic solvent and the other solution may include RNA in an aqueous solvent.

[0044] At block **320**, measurements of physical properties of the particles, e.g., size, polydispersity and/or concentration of LNP-RNA particles of the formulations formed by the mixing step in block **310** are determined, for example, by an inline measurement apparatus such as a particle analyzer, e.g., an ultraDAWN™ particle analyzer or DynaPro™ ZetaStar™ light scattering instrument by Wyatt Technology, but not limited thereto. This provides immediate feedback to a user, e.g., via a computer display or other visual, audio, and/or tactile communication device, regarding the formation of nanoparticles based on the conditions provided by the computer.

[0045] At block **330**, each sample is collected by the sample collector from the particle analyzer and in doing so is output to a collection vial or well in a well plate or other vessel. The sample collector is constructed and arranged to accommodate a wide range of adapter racks and related collection vessels. In some embodiments, the samples that are output of the sample collector can be tested, e.g., periodically remeasured, following a ripening period by a light scattering instrument, autosampler, plate reader, or other instrument for remeasuring particle size, concentration, and so on.

[0046] FIG. **4** depicts a flowchart of a method **400**, in accordance with an embodiment. In describing the method **400**, reference is made to components of a system of FIG. **1** or **2**. The steps in the method **400** may be performed without human intervention, i.e., using only the system components and in some embodiments, including a robotic apparatus.

[0047] At block **410**, a special-purpose computer generates and outputs at least one command to a microfluidic mixing apparatus to mix at least two solutions. The command may correspond to a series of mixing parameters and the mixer may perform a mixing operation according to the parameters. In some embodiments, one of the solutions includes encapsulating molecules in an organic solvent and the other solution includes payload molecules in an aqueous solvent, resulting in formulations of nano-capsules encapsulating the payload molecules, e.g., RNA. For example, the computer can instruct the mixing apparatus to form nanoparticles, e.g., LNPs encapsulating RNA (LNP-RNA), by mixing a payload and lipid packets comprised of cationic and neutral lipids in respective solutions.

[0048] At block **420**, the special-purpose computer generates and outputs at least one command to a sample collection apparatus, for example, a fraction collector, to direct the series of formulations to the fraction collector such that each formulation is dispensed to a distinct receptacle, e.g., a vial or well in a well plate, corresponding to the corresponding mixing parameters, e.g., translate a receptacle to receive dispensed fluid from the mixer.

[0049] At block **430**, the special-purpose computer generates and outputs at least one command to a particle analyzer to analyze LNP-RNA particles, nano-capsules, or the like in each formulation. The particle analyzer can be an ultraDAWN™ particle analyzer or DynaPro™ ZetaStar™ light scattering instrument by Wyatt Technology, but not limited thereto.

[0050] In some embodiments, in response to the computer commands, the instrument, e.g., light scattering instrument, autosampler, plate reader, or other instrument for remeasuring particle size, concentration, and so on, performs measurements of physical properties of the LNP-RNA particles or nano-capsules, such as particle size, concentration, and so on.

[0051] At block **450**, measurements corresponding to mixing parameters are displayed, for example, at a computer display or other I/O device of the computer system **130** of FIG. **1**.

[0052] FIG. **6** depicts an example of a system **600** configured for condition screening with inline dilution, in accordance with an embodiment. The system **600** may allow for the screening of different buffer conditions so that a sample can have a desired state, while providing for inline dilution to enhance nanoparticle stability.

[0053] In some embodiments, the system **600** includes microfluidic mixing system **610**. The microfluidic mixing system **610** may include microfluidic mixers selected from the group consisting of microfluidic mixing chips, T-junction mixers, Y-junction mixers, and augmented T-junction mixers. In some embodiments, the microfluidic mixing system **610** includes a first microfluidic chip **611** serially connected to a second microfluidic chip **612** in series. A first pump **613** and a second pump **614** are coupled via tubing or other flow path to the inlets of the first microfluidic chip **611**. The outlet of the first microfluidic chip **611** and a third pump **614** are coupled to the inlets of the second microfluidic chip **612**. In some embodiments, the first pump **613**, second pump **614**, and/or third pump **615** are syringe pumps. In some embodiments, one or more of the first through third pumps **615** can be continuous pumps selected from the group consisting of peristaltic pumps, pressure-driven pumps, piston pumps, and diaphragm pumps. The first pump **613** can provide a lipid solution to the first inlet of the first microfluidic chip **611** and the second pump **614** can provide an RNA solution to the second inlet of the first microfluidic chip **611**, which can produce a formulation of lipid nanoparticles (LNPs) encapsulating the RNA that is output to the first inlet of the second microfluidic chip **612**. In some experiments, the total flow rate (mL/min) of the first and second pumps **613**, **614** may be 4, 6, 8, 10, 12, 14, or 16 mL/min, but not limited thereto. The flow rate ratio of the first pump flow rate to second pump flow rate may be 1:3, 1:4, 1:5, or 1:6 but not limited thereto. The volume of each solution may be 0.5 mL, but not limited thereto. The system **600** can produce 16 conditions per run, but not limited thereto.

[0054] The third pump **615** can provide a source of PBS or the like to the second inlet of the second microfluidic chip **612** to provide an inline buffer dilution, which can be performed for stability enhancement or the like.

[0055] The second microfluidic chip **612** outputs a mixture of the outputs of the first through third pumps **613**-**615** to a measurement apparatus **620**, for example, similar to or the same as the light scattering instrument **215** of FIG. **2**.

[0056] FIG. **7** depicts an example of a system **700** configured for condition screening with buffer variation, in accordance with an embodiment. The system **700** includes a microfluidic mixing system **710**, which in some embodiments includes a first microfluidic mixer **712**, receiving solutions from a first pump **713** and an output of a second microfluidic mixer **716**, which in turn have two inlets for receiving solutions from a second pump **714** and a third pump **715**, respectively. In some embodiments, the first pump **713**, second pump **714**, and/or third pump **715** are syringe pumps. The first pump **713** can provide a lipid solution to the first inlet of the first mixer **712**. The second pump **714** can provide a first RNA solution to the first inlet of the second mixer **716**. In some embodiments, the first RNA solution includes a first buffer, for example, 50 mM NaCl. The third pump **715** can provide a second RNA solution to the second inlet of the second mixer **716**. In some embodiments, the second RNA solution includes a second buffer, for example, 250 mM NaCl. The second mixer **716** can mix the received volumes of RNA solution from the first and second inlets and output the mixed volumes to the second inlet of the first mixer **716**.

[0057] Accordingly, the microfluidic mixer (or mixing device or mixing chip) **712** can produce a formulation of lipid nanoparticles (LNPs) encapsulating the RNA. In some experiments, the volumes, total flow rate (mL/min), and flow rate ratio are similar to those provided by the system **600** of FIG. **6**. In some experiments, the flow rates provided by the second pump **714** and third pump **715** may vary, for example, to achieve 50, 100, 150, 200, or 250 mM NaCl. The volume of each solution may be 0.5 mL, but not limited thereto. The system **700** can produce 120 conditions per run, but not limited thereto.

[0058] The mixer **712** outputs a mixture of the outputs of the first through third pumps **713**-**715** to a measurement apparatus **720**, for example, similar to or the same as the light scattering instrument **215** of FIG. **2**.

[0059] FIG. **8** depicts an example of a system **800** comprising a microfluidic mixing system **810** and Multi-Angle Light Scattering (MALS) detection instrument **820** configured for generating volumes larger than those offered by the configurations of FIGS. **6** and **7**, in accordance with an embodiment. The system **800** includes a first pump **813** coupled via tubing or other flow path element to a first inlet of a microfluidic chip **811**. The system **800** also includes a three-port union **812**. A second pump **814** and third pump **815** are coupled to the two inlets of the union **812**. The union outlet is coupled to a second inlet of the microfluidic chip **811**. Accordingly, the second pump **814** and third pump **815** can operate as a reciprocating pump such that while pump **814** dispenses at a dispense flow rate, pump **814** fills at a fill flow rate that is equal to or faster than the dispense rate, and when pump **814** has emptied the operation reverses: pump **815** dispenses at the dispense flow rate and pump **814** fills at the fill flow rate, and this alternating (reciprocating) operation continues as many times as necessary. In other words, some embodiments may include at least three pumps that are configured and instructed to deliver solutions to a microfluidic mixer such that a first pump of the at least three pumps delivers the solution with encapsulating molecules, wherein second and third pumps of the at least three pumps deliver a solution containing payload molecules, and the apparatus is controlled to operate the second and third pumps as a reciprocating pump such that while one of the second and third pumps dispenses the other of the second and third pumps loads, then the second and third pumps switch so that while the other of the second and the third pumps dispenses the one of the second and third pumps loads in order to increase a quantity of nano capsules produced before the first pump runs out of encapsulating molecule solution. In some embodiments, the first pump **613**, second pump **614**, and/or third pump **615** are syringe pumps. The first pump **813** can provide a lipid solution to the first inlet of the microfluidic chip **811**. The second pump **814** can provide a first RNA and buffer solution to the first inlet of the union **812** and the third pump **814** can provide a second RNA and buffer solution to the second inlet of the union **812**. In some experiments, the total flow rate 10 mL/min, the flow rate ratio is 1:4, the volume: 10 mL, and 10 repeats can be performed for a total of 100 mL at 1 condition per run, but not limited thereto.

[0060] FIG. **9** depicts an example of a system **900** configured for condition screening of small volumes, in accordance with an embodiment. The system **900** includes a three-inlet mixer **912**, including three inlets for receiving solutions from a first pump **913**, a second pump **914**, and a third pump **915**, respectively. In some embodiments, the first pump **913**, second pump **914**, and/or third pump **915** are syringe pumps. The first pump **913** can provide a lipid solution to the first inlet of the mixer **912**. The second pump **914** can provide an RNA and buffer solution to the second inlet of the mixer **912**. In some embodiments, the first RNA solution includes a first buffer source, for example, 50 mM NaCl. The third pump **915** can provide a source of PBS or the like to the third inlet of the mixer **912** following the production of nanoparticles by mixing the solutions, supplied by pumps **913** and **914**, in the mixer **912**.

[0061] The mixer **912** can produce a formulation of lipid nanoparticles (LNPs) encapsulating the RNA from the two inputs supplied by pumps **913** and **914**. In some experiments, the total flow rate (mL/min) and flow rate ratio are similar to those provided by the system **700** of FIG. **7**, but the volume is smaller, for example, 0.05 mL. Upon completing the formulation of nanoparticles, the third pump **915** is used to push a volume of PBS or the like through the outlet tubing and particle analyzer **920** in order for the nanoparticles to reach the sample collector.

[0062] FIG. **10** depicts an example of a system **1000** configured for condition and lipid mixture screening, in accordance with an embodiment. The system **1000** includes a first mixer **1016**, receiving solutions from a first pump **1013** and a second pump **1014**, and a second mixer receiving the output from mixer **1016** a third pump **1015**. In some embodiments, the first pump **1013**, second pump **1014**, and/or third pump **1015** are syringe pumps. In some embodiments, the first pump **1013** includes a multi-port valve, e.g., an 8-port valve, for providing up to 6 lipid solutions to the first inlet of the mixer **1012**. The second pump **1014** can provide an RNA and buffer solution to the second inlet of the mixer **1012**. In some embodiments, the first RNA solution includes a first buffer source, for example, 50 mM NaCl. The third pump **1015** can provide a source of PBS or the like to the mixer **1012** to provide an inline buffer dilution, which can enhance stability of the nanoparticle formulation or the like.

[0063] The mixer **1012** can produce a formulation of lipid nanoparticles (LNPs) encapsulating the RNA from the three inputs. In some experiments, the volumes, total flow rate (mL/min), and flow rate ratio are similar to those provided by a system of FIGS. **6**-**9**. However, the system **1000** can provide up to 96 different samples per run, or 16 conditions per run for each of 6 lipid mixtures.

[0064] FIG. **11** depicts an example of a system **1100** configured for condition screening with an inline buffer exchange, in accordance with an embodiment. The system **1100** includes a microfluidic mixing chip **1111** serially connected to a hollow-fiber (HF) cartridge **1112** for TFF. A first pump **1113** and a second pump **1114** are coupled via tubing or other flow path to the inlets of the microfluidic chip

1111. The outlet of the first microfluidic chip **1111** and a third pump **1114** are coupled to the inlets of the HF cartridge **1112**. In some embodiments, the first pump **1113**, second pump **1114**, and/or third pump **1115** are syringe pumps. The first pump **1113** can provide a lipid solution to the first inlet of the microfluidic chip **1111** and the second pump **1114** can provide an RNA solution to the second inlet of the microfluidic chip **1111**, which can produce a formulation of lipid nanoparticles (LNPs) encapsulating the RNA that is output to the first inlet of the HF cartridge **1112**, which may have a second outlet to a waste outlet or the like for removing solvent or the like from the input to the cartridge **1112**. The first and second pumps may provide formulation conditions such as total flow rate, e.g., 4, 6, 8, 10 mL/min, etc. and the third pump provides for a buffer exchange, for example, a flow rate of 80% (which is equal to 4 times the combined flow rates of the first and second pumps. In some experiments, the volumes, total flow rate (mL/min), and flow rate ratio are similar to those provided by a system of FIGS. **6-10**. However, the third pump **1115** can provide a source of PBS or the like to the second inlet of the second microfluidic chip **612** to provide an inline buffer exchange and dilution for enhancing formulation stability, where the third pump flow rate equals four (4) times the combined first and second pump flow rates.

[0065] FIG. **13** depicts a computer system **1300** in accordance with an exemplary embodiment. In an exemplary embodiment, the computer system **1300** is a standalone computer system, a network of distributed computers, or a cloud computing node server. In some embodiments, the computer system **1300** can perform some or all of the method **400** of FIG. **4** and/or method **500** of FIG. **5**. Computer system **1300** is only one example of a computer system and is not intended to suggest any limitation as to the scope of use or functionality of embodiments of the present disclosure. Regardless, computer system **1300** is capable of being implemented to perform and/or performing any of the functionality/operations of the present disclosure.

[0066] Computer system **1300** includes a computer system/server **1312**, which is operational with numerous other general purpose or special purpose computing system environments or configurations. Examples of well-known computing systems, environments, and/or configurations that may be suitable for use with computer system/server **1312** include, but are not limited to, personal computer systems, server computer systems, thin clients, thick clients, handheld or laptop devices, multiprocessor systems, microprocessor-based systems, set top boxes, programmable consumer electronics, network PCs, minicomputer systems, mainframe computer systems, and distributed cloud computing environments that include any of the above systems or devices.

[0067] Computer system/server **1312** may be described in the general context of computer system-executable instructions, such as program modules, being executed by a computer system. Generally, program modules may include routines, programs, objects, components, logic, and/or data structures that perform particular tasks or implement particular abstract data types. Computer system/server **1312** may be practiced in distributed cloud computing environments where tasks are performed by remote processing devices that are linked through a communications network. In a distributed cloud computing environment, program modules may be located in both local and remote computer system storage media including memory storage devices.

[0068] As shown in FIG. **13**, the components of computer system/server **1312** may include, but are not limited to, one or more processors or processing units **1316**, a system memory **1328**, and a bus **1318** that couples various system components including system memory **1328** to processor **1316**. The computer system **130** of FIG. **1** may include some or all of the components described with reference to the computer system **1300**.

[0069] Bus **1318** represents one or more of any of several types of bus structures, including a memory bus or memory controller, a peripheral bus, an accelerated graphics port, and a processor or local bus using any of a variety of bus architectures. By way of example, and not limitation, such architectures include Industry Standard Architecture (ISA) bus, Micro Channel Architecture (MCA) bus, Enhanced ISA (EISA) bus, Video Electronics Standards Association (VESA) local bus, and Peripheral Component Interconnects (PCI) bus.

[0070] Computer system/server **1312** typically includes a variety of computer system readable media. Such media may be any available media that is accessible by computer system/server **1312**, and includes both volatile and non-volatile media, removable and non-removable media.

[0071] System memory **1328** can include computer system readable media in the form of volatile memory, such as random access memory (RAM) **1330** and/or cache memory **1332**.

[0072] Computer system/server **1312** may further include other removable/non-removable, volatile/non-volatile computer system storage media. By way of example only, storage system **1334** can be provided for reading from and writing to a non-removable, non-volatile magnetic media (not shown and typically called a "hard drive"). Although not shown, a magnetic disk drive for reading from and writing to a removable, non-volatile magnetic disk (e.g., a "floppy disk"), and an optical disk drive for reading from or writing to a removable, non-volatile optical disk such as a CD-ROM, DVD-ROM or other optical media can be provided. In such instances, each can be connected to bus **1318** by one or more data media interfaces. As will be further depicted and described below, memory **1328** may include at least one program product having a set (e.g., at least one) of program modules that are configured to carry out the functions/operations of embodiments of the disclosure.

[0073] Program/utility **1340**, having a set (at least one) of program modules **1342**, may be stored in memory **1328** by way of example, and not limitation. Exemplary program modules **1342** may include an operating system, one or more application programs, other program modules, and program data. Each of the operating system, one or more application programs, other program modules, and program data or some combination thereof, may include an implementation of a networking environment. Program modules **1342** generally carry out the functions and/or methodologies of embodiments of the present disclosure.

[0074] Computer system/server **1312** may also communicate with one or more external devices **1314** such as a keyboard, a pointing device, a display **1324**, one or more devices that enable a user to interact with computer system/server **1312**, and/or any devices (e.g., network card, modem, etc.) that enable computer system/server **1312** to communicate with one or more other computing devices. Such communication can occur via Input/Output (I/O) interfaces **1322**. Still yet, computer system/server **1312** can communicate with one or more networks such as a local area network (LAN), a general wide area network (WAN), and/or a public network (e.g., the Internet) via network adapter **1320**. As depicted, network adapter **1320** communicates with the other components of computer system/server **1312** via bus **1318**. It should be understood that although not shown, other hardware and/or software components could be used in conjunction with computer system/server **1312**. Examples include, but are not limited to microcode, device drivers, redundant processing units, external disk drive arrays, RAID systems, tape drives, and data archival storage systems.

[0075] The present disclosure may be a system, a method, and/or a computer program product. The computer program product may include a computer readable storage medium (or media) having computer readable program instructions thereon for causing a processor to carry out aspects of the present disclosure.

[0076] The computer readable storage medium can be a tangible device that can retain and store instructions for use by an instruction execution device. The computer readable storage medium may be, for example, but is not limited to, an electronic storage device, a magnetic storage device, an optical storage device, an electromagnetic storage device, a semiconductor storage device, or any suitable combination of the foregoing. A non-exhaustive list of more specific examples of the computer readable storage medium includes the following: a portable computer diskette, a hard disk, a random access memory (RAM), a read-only memory (ROM), an erasable programmable read-only memory (EPROM or Flash memory), a static random access memory (SRAM), a portable compact disc read-only memory (CD-ROM), a digital versatile disk (DVD), a memory stick, a floppy disk, a mechanically encoded device such as punch-cards or raised structures in a groove having instructions recorded thereon, and any suitable combination of the foregoing. A computer readable storage medium, as used herein, is not to be construed as being transitory signals per se, such as radio waves or other freely propagating electromagnetic waves, electromagnetic waves propagating through a waveguide or other transmission media (e.g., light pulses passing through a fiber-optic cable), or electrical signals transmitted through a wire.

[0077] Computer readable program instructions described herein can be downloaded to respective computing/processing devices from a computer readable storage medium or to an external computer or external storage device via a network, for example, the Internet, a local area network, a wide area network and/or a wireless network. The network may comprise copper transmission cables, optical transmission fibers, wireless transmission, routers, firewalls, switches, gateway computers and/or edge servers. A network adapter card or network interface in each computing/processing device receives computer readable program instructions from the network and forwards the computer readable program instructions for storage in a computer readable storage medium within the respective computing/processing device.

[0078] Computer readable program instructions for carrying out operations of the present disclosure may be assembler instructions, instruction-set-architecture (ISA) instructions, machine instructions, machine dependent instructions, microcode, firmware instructions, state-setting data, or either source code or object code written in any combination of one or more programming languages, including an object oriented programming language such as Smalltalk, C++ or the like, and conventional procedural programming languages, such as the "C" programming language or similar programming languages. The computer readable program instructions may execute entirely on the user's computer, partly on the user's computer, as a stand-alone software package, partly on the user's computer and partly on a remote computer or entirely on the remote computer or server. In the latter scenario, the remote computer may be connected to the user's computer through any type of network, including a local area network (LAN) or a wide area network (WAN), or the connection may be made to an external computer (for example, through the Internet using an Internet Service Provider). In some embodiments, electronic circuitry including, for example, programmable logic circuitry, field-programmable gate arrays (FPGA), or programmable logic arrays (PLA) may execute the computer readable program instructions by utilizing state information of the computer readable program instructions to personalize the electronic circuitry, in order to perform aspects of the present disclosure.

[0079] Aspects of the present disclosure are described herein with reference to flowchart illustrations and/or block diagrams of methods, apparatus (systems), and computer program products according to embodiments of the disclosure. It will be understood that each block of the flowchart illustrations and/or block diagrams, and combinations of blocks in the flowchart illustrations and/or block diagrams, can be implemented by computer readable program instructions.

[0080] These computer readable program instructions may be provided to a processor of a general purpose computer, special purpose computer, or other programmable data processing apparatus to produce a machine, such that the instructions, which execute via the processor of the computer or other programmable data processing apparatus, create means for implementing the functions/acts specified in the flowchart and/or block diagram block or blocks. These computer readable program instructions may also be stored in a computer readable storage medium that can direct a computer, a programmable data processing apparatus, and/or other devices to function in a particular manner, such that the computer readable storage medium having instructions stored therein comprises an article of manufacture including instructions which implement aspects of the function/act specified in the flowchart and/or block diagram block or blocks.

[0081] The computer readable program instructions may also be loaded onto a computer, other programmable data processing apparatus, or other device to cause a series of operational steps to be performed on the computer, other programmable apparatus or other device to produce a computer implemented process, such that the instructions which execute on the computer, other programmable apparatus, or other device implement the functions/acts specified in the flowchart and/or block diagram block or blocks.

[0082] The flowchart and block diagrams in the figures illustrate the architecture, functionality, and operation of possible implementations of systems, methods, and computer program products according to various embodiments of the present disclosure. In this regard, each block in the flowchart or block diagrams may represent a module, segment, or portion of instructions, which comprises one or more executable instructions for implementing the specified logical function(s). In some alternative implementations, the functions noted in the block may occur out of the order noted in the figures. For example, two blocks shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved. It will also be noted that each block of the block diagrams and/or flowchart illustration, and combinations of blocks in the block diagrams and/or flowchart illustration, can be implemented by special purpose hardware-based systems that perform the specified functions or acts or carry out combinations of special purpose hardware and computer instructions.

[0083] The descriptions of the various embodiments of the present disclosure have been presented for purposes of illustration, but are not intended to be exhaustive or limited to the embodiments disclosed. Many modifications and variations will be apparent to those of ordinary skill in the art without departing from the scope and spirit of the described embodiments. The terminology used herein was chosen to explain the principles of the embodiments, the practical application or technical improvement over technologies found in the marketplace, or to enable others of ordinary skill in the art to understand the embodiments disclosed herein.

What is claimed is:

1. A computer-implemented method, comprising:

receiving, by a computer system, a series of mixing parameters;

transmitting, by the computer system, a series of commands corresponding to the series of mixing parameters to a microfluidic mixing system to mix a series of at least two solutions, wherein one of the solutions includes lipids in an organic solvent and the other of the solutions includes ribonucleic acid (RNA) in an aqueous solvent; and

generating in response a plurality of formulations of lipid nanoparticles (LNPs) encapsulating the RNA, LNP-RNA particles, according to the series of mixing parameters.

2. The computer-implemented method of claim **1**, further comprising:

transmitting, by the computer system, a command to direct the plurality of formulations to a sample collector such that each of the formulations is dispensed to a distinct receptacle corresponding to corresponding mixing parameters.

3. The computer-implemented method of claim **1**, further comprising:

transmitting, by the computer system, a command to a particle analyzer for performing inline analytics to analyze LNP-RNA particles in each of the formulations, resulting in measurements of physical properties of the LNP-RNA particles.

4. The computer-implemented method of claim **3**, further comprising:

storing, by the computer system, the measurements in a database corresponding to the mixing parameters.

5. The computer-implemented method of claim **3**, further comprising:

displaying, by the computer system, on a display the measurements corresponding to the mixing parameters.

6. The computer-implemented method of claim **3**, wherein the particle analyzer is selected from the group consisting of a Multi-Angle Light Scattering (MALS) detection instrument, a dynamic light scattering (DLS) instrument, and an electrophoretic light scattering (ELS) instrument.

7. The computer-implemented method of claim **1**, wherein the transmitting comprises

transmitting, by the computer system, the series of commands corresponding to the series of mixing parameters to a microfluidic mixer to mix a series of at least two solutions, wherein one of the solutions includes encapsulating molecules in an organic solvent and the other of the solutions includes payload molecules in an aqueous solvent.

8. A computer-implemented method, comprising:

receiving, by a computer system, a series of mixing parameters;

transmitting, by the computer system, a series of commands corresponding to the series of mixing parameters to a microfluidic mixer to mix a series of at least two solutions, wherein one of the solutions includes encapsulating molecules in an organic solvent and the other of the solutions includes payload molecules in an aqueous solvent, resulting in formulations of nano-capsules encapsulating the payload molecules according to the series of mixing parameters; and

transmitting, by the computer system, a command to direct the formulations to a sample collector such that each of the formulations is dispensed to a distinct receptacle corresponding to corresponding mixing parameters.

9. The computer-implemented method of claim **8**, further comprising

transmitting, by the computer system, a command to a particle analyzer to analyze nano-capsules in each of the formulations, resulting in measurements of physical properties of the nano-capsules.

10. The computer-implemented method of claim **9**, further comprising:

storing, by the computer system, the measurements in a database corresponding to the mixing parameters.

11. The computer-implemented method of claim **9**, further comprising:

displaying, by the computer system, on a display the measurements corresponding to the mixing parameters.

12. An apparatus comprising:

a microfluidic mixer that mixes a series of at least two solutions, wherein one of the solutions includes encapsulating molecules in an organic solvent and the other of the solutions includes payload molecules in an aqueous solvent, resulting in formulations; and

a sample collector to dispense each of the formulations to a distinct receptacle corresponding to corresponding mixing parameters and to translate a receptacle to receive dispensed fluid from the mixer.

13. The apparatus of claim **12** further comprising:

a flow-through particle analyzer to analyze lipid nanoparticles (LNPs) encapsulating ribonucleic acid (RNA), LNP-RNA particles, in each of the formulations, resulting in measurements of physical properties of the LNP-RNA particles.

14. The apparatus of claim **12**, further comprising at least three pumps, wherein the microfluidic mixer includes a first microfluidic mixer and a second microfluidic mixer and two of the at least three pumps are configured and instructed to deliver the encapsulating molecules including lipids and the payload molecules, respectively, to the first microfluidic mixer in order to produce nanocapsules, and wherein the nanocapsules are further combined in the second microfluidic mixer with a diluent (PBS) delivered by a third pump of the at least three pumps in order to stabilize the nanocapsules.

15. The apparatus of claim **12**, further comprising at least three pumps, wherein the microfluidic mixer includes a first microfluidic mixer and a second microfluidic mixer and two of the at least three pumps are configured and instructed to deliver the encapsulating molecules including lipids and the payload molecules, respectively, to the first microfluidic mixer in order to produce nanocapsules, and wherein the nanocapsules are further combined in a hollow fiber cartridge with a diluent delivered by a third pump of the at least three pumps in order to remove organic solvent and thus stabilize the nanocapsules.

16. The apparatus of claim **12**, further comprising at least three pumps, wherein the at least three pumps are configured and instructed to deliver solutions to the microfluidic mixer such that a first pump of the at least three pumps delivers the solution with encapsulating molecules including lipids, a second pump of the at least three pumps delivers a solution containing payload molecules in a first aqueous buffer and the third pump delivers a solution containing the payload molecules in a second aqueous buffer, and the apparatus is controlled to vary the ratio between the second pump and the third pump in order to test the effect of varying aqueous buffers on final nanocapsules.

17. The apparatus of claim **12**, further comprising at least three pumps, wherein the at least three pumps are configured and instructed to deliver solutions to the microfluidic mixer such that a first pump of the at least three pumps delivers the solution with encapsulating molecules, wherein second and third pumps of the at least three pumps deliver a solution containing payload molecules, and the apparatus is controlled to operate the second and third pumps as a reciprocating pump such that while one of the second and third pumps dispenses, the other of the second and third pumps loads, then the second and third pumps switch so that while the other of the second and the third pumps dispenses, the one of the second and third pumps loads in order to increase a quantity of nanocapsules produced before the first pump runs out of an encapsulating molecule solution.

18. The apparatus of claim **12**, further comprising at least three pumps, wherein first and second pumps of the at least three pumps are configured and instructed to deliver the encapsulating molecules and the payload molecules, respectively, to the microfluidic mixer in order to produce a small quantity of nanocapsules, such that the nanocapsules are initially contained within a volume of capillary tubing, and the nanocapsules are pushed out of the system to a sample collector with an aqueous solution delivered by a third pump of the at least three pumps.

19. The apparatus of claim **12**, further comprising a pump configured with a solution selection valve that can be controlled to draw from any of a set of solutions of encapsulating molecules wherein the set of solutions comprises different compositions of encapsulating molecules in an organic solvent.

20. The apparatus of claim **12**, wherein the microfluidic mixing device comprises more than three pumps.

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