

SUMMARY

[0008] Presented herein are data supporting the feasibility of a method for preparing a high concentration, liquid composition comprising an oligonucleotide compound. As detailed herein, the method achieves preparation of liquid compositions comprising the oligonucleotide compound at concentrations as high as about 150 mg/mL, and, in various instances, the method achieves even higher concentrations of the oligonucleotide compound. As described herein, the method achieves, for instance, a liquid composition comprising an oligonucleotide compound at a concentration greater than about 150 mg/mL, greater than about 160 mg/mL, greater than about 170 mg/mL, greater than about 180 mg/mL, greater than about 190 mg/mL, greater than about 200 mg/mL, greater than about 210 mg/mL, greater than about 220 mg/mL, or greater than about 230 mg/mL.

[0009] Without being bound to a particular theory, the achievement of such high concentration, liquid compositions are, at least in part, attributed to the solution in which the oligonucleotide compound exists during ultrafiltration, which, in exemplary aspects, relates to the diafiltration (DF) solution used in the method. Without being bound by theory, the solution in which the oligonucleotide compound exists during ultrafiltration, or the DF solution used for diafiltration, comprises one or more salts which interact with the oligonucleotide compound in a way which stabilizes the oligonucleotide compound and/or increases its hydrodynamic diameter. Without being bound to a particular theory, the salt(s) of the solution, e.g., DF solution, interact(s) with the oxygen atoms and/or sulfur atoms of the oligonucleotide compound via, e.g., hydrogen bonds, metal coordination, electrostatic interactions. Without being bound to a particular theory, the more interactions made between the oligonucleotide compound and the salt(s), the higher the concentration of the oligonucleotide compound may be achieved. Such interactions are associated with the absence of or a minimization of fouling of the membrane used in the method.

[0010] Accordingly, provided herein are methods for preparing a high concentration, liquid composition comprising an oligonucleotide compound, wherein the liquid composition comprises the oligonucleotide compound at a concentration greater than about 150 mg/mL, greater than about 160 mg/mL, greater than about 170 mg/mL, greater than about 180 mg/mL, greater than about 190 mg/mL, greater than about 200 mg/mL, greater than about 210 mg/mL, greater than about 220 mg/mL. In exemplary examples, the method of preparing the high concentration, liquid composition comprises: (a) preparing an oligonucleotide compound in a first solution comprising one or more salts, wherein the concentration of the oligonucleotide compound in the

first solution is 140 mg/mL or less, and the total salt concentration of the first solution is about 25 mM to about 800 mM, and (b) concentrating by ultrafiltration the first solution to obtain a high concentration, liquid composition comprising the oligonucleotide compound at a concentration of about 150 mg/mL or greater. In various examples, the first solution is prepared by diafiltration with a DF solution. In various examples, the first solution is the same as the DF solution except that the first solution comprises the oligonucleotide compound and the DF solution does not comprise the oligonucleotide compound. In various instances, the diafiltration achieves an exchange of a starting solution comprising the oligonucleotide compound at about 140 mg/mL or less into a DF solution comprising one or more salts and having a total salt concentration of 25 mM to about 800 mM. Accordingly, in exemplary examples, the method of preparing the high concentration liquid composition comprises: (a) exchanging by diafiltration an oligonucleotide compound in a starting solution into a DF solution to obtain an intermediate solution, wherein the concentration of the oligonucleotide compound in the starting solution is 140 mg/mL or less, and the DF solution comprises one or more salts, and (b) concentrating by ultrafiltration the oligonucleotide compound in the intermediate solution to obtain a high concentration, liquid composition, wherein the concentration of the oligonucleotide compound in the high concentration, liquid composition is about 150 mg/mL or greater. In exemplary instances, the total salt concentration of the DF solution is about 25 mM to about 800 mM. Optionally, the total salt concentration of the DF solution is about 25 mM to about 500 mM. In exemplary instances, the method comprises continuous diafiltration and/or ultrafiltration by tangential flow filtration. In various examples, the method employs a polyethersulfone (PES) membrane or a stabilized cellulose membrane for the diafiltration and/or ultrafiltration. Optionally, the membrane has a molecular weight cut-off (MWCO) of less than 10 kDa, and in exemplary instances, the MWCO is about 5 kDa or about 3 kDa.

[0011] Also provided herein are methods of concentrating by ultrafiltration a first solution comprising a low concentration of an oligonucleotide compound to obtain a second solution comprising a high concentration of the oligonucleotide compound, wherein the oligonucleotide compound concentration of the second solution is greater than about 150 mg/mL, greater than about 160 mg/mL, greater than about 170 mg/mL, greater than about 180 mg/mL, greater than about 190 mg/mL, greater than about 200 mg/mL, greater than about 210 mg/mL, greater than about 220 mg/mL. In exemplary examples, the oligonucleotide compound concentration of the first solution is less than about 140 mg/mL, less than about 130 mg/mL, less than about 120 mg/mL, less than about 110 mg/mL, less than about 100 mg/mL, less than about 90 mg/mL, less than about 80 mg/mL, less than about 70 mg/mL, less than about 60 mg/mL, or less than

about 50 mg/mL. In various examples, the second solution is a retentate obtained upon ultrafiltration. In various examples, the retentate is achieved after 1 or 2 hours of ultrafiltration. In exemplary instances, the oligonucleotide compound is double stranded. In exemplary instances, the total salt concentration of the first solution is about 25 mM to about 800 mM. Optionally, the total salt concentration of the first solution is about 25 mM to about 500 mM or about 25 mM to about 250 mM. In various examples, the first solution comprises only one salt. Optionally, the one salt is an inorganic salt, such as, any of the inorganic salts described herein. In various examples, the first solution does not comprise any acetate. In exemplary instances, the method of concentrating is carried out after diafiltration with a DF solution. In various examples, the first solution is the same as the DF solution except that the first solution comprises the oligonucleotide compound and the DF solution does not comprise the oligonucleotide compound. In various instances, the diafiltration is carried out with a DF solution comprising one or more salts and the total salt concentration of the DF solution is about 25 mM to about 800 mM. Optionally, the total salt concentration of the DF solution is about 25 mM to about 500 mM or about 25 mM to about 250 mM. In exemplary examples, the method of concentrating is carried out before diafiltration with a DF solution. In various instances, the oligonucleotide compound is a double-stranded oligonucleotide compound, optionally, an siRNA.

[0012] Further provided herein is a method of preparing a solution API comprising an oligonucleotide compound API, wherein the oligonucleotide compound API is present in the solution at a concentration greater than 150 mg/mL. In various examples, the oligonucleotide compound API is double-stranded, optionally, an siRNA. In exemplary examples, the method of preparing a solution API comprises a presently disclosed method of concentrating by ultrafiltration a first solution comprising a low concentration of an oligonucleotide compound to obtain a second solution comprising a high concentration of the oligonucleotide compound. In various instances, the method of preparing a solution API comprises (i) synthesizing the oligonucleotide compound, or a strand thereof, by, e.g., solid phase synthesis, (ii) carrying out one or more of a chromatography, a diafiltration, and an annealing, and (iii) concentrating by ultrafiltration in accordance with a presently disclosed method of concentrating by ultrafiltration a first solution comprising a low concentration of an oligonucleotide compound to obtain a second solution comprising a high concentration of the oligonucleotide compound. In various examples, the oligonucleotide compound concentration of the second solution is greater than about 150 mg/mL, greater than about 160 mg/mL, greater than about 170 mg/mL, greater than about 180 mg/mL, greater than about 190 mg/mL, greater than about 200 mg/mL, greater than about 210 mg/mL, greater than about 220 mg/mL, and the oligonucleotide compound concentration of the

first solution is less than about 140 mg/mL, less than about 130 mg/mL, less than about 120 mg/mL, less than about 110 mg/mL, less than about 100 mg/mL, less than about 90 mg/mL, less than about 80 mg/mL, less than about 70 mg/mL, less than about 60 mg/mL, or less than about 50 mg/mL. In various examples, the second solution is a retentate obtained upon ultrafiltration. In various examples, the retentate is achieved after 1 or 2 hours of ultrafiltration. In exemplary examples of the method of preparing a solution API comprising an oligonucleotide compound API, the oligonucleotide API is not lyophilized at any point during the method. Advantageously, the method of preparing the solution API is a method which lacks any lyophilization. Accordingly, the method of preparing the solution API of the present disclosure is free of a lyophilization and provides a more time- and cost-efficient way of preparing an oligonucleotide compound API. In various instances, the solution API prepared by the presently disclosed method is directly used for preparing a solution DP comprising the oligonucleotide compound API. Optionally, the solution API is sterile filtered and filled into vials or pre-filled syringes or autoinjectors. Accordingly, a method of producing a DP, e.g., a solution DP, comprising a solution API comprising an oligonucleotide compound API is provided. In various examples, the method of producing the DP lacks any lyophilization. In various examples of the method of producing the DP, lyophilization of the oligonucleotide compound API is not carried out at any time during the method.

[0013] In exemplary examples, the method of the present disclosure leads to a high concentration, liquid composition, wherein the concentration of the oligonucleotide compound in the high concentration, liquid composition is about 150 mg/mL or greater. In exemplary examples, the high concentration, liquid composition obtained by the presently disclosed method comprises greater than 80% of the oligonucleotide compound of the starting solution. In exemplary instances, the method achieves a 70% or an 80% recovery of the oligonucleotide compound of the starting solution. In exemplary examples, membrane flux is substantially maintained during the method, e.g., during the diafiltration and/or ultrafiltration. In various instances, membrane flux is substantially maintained for at least 6 hours, at least 8 hours, or greater than 9 hours. In exemplary examples, little to no membrane fouling occurs during the method, e.g., during the diafiltration and/or ultrafiltration. In exemplary instances, a high concentration, liquid composition comprising the oligonucleotide compound at a concentration of about 150 mg/mL or greater is obtained, and the membrane flux decreases by not more than 50% of the steady state membrane flux, which optionally is maintained for at least 4 hours or more.

[0013a] In one aspect, the present disclosure provides for a method of preparing a high concentration, liquid composition comprising an oligonucleotide compound, said method comprising: a. exchanging by diafiltration an oligonucleotide compound in a starting solution into a diafiltration (DF) solution to obtain an intermediate solution, wherein the concentration of the oligonucleotide compound in the starting solution is 140 mg/mL or less and the DF solution comprises one or more salts and the total salt concentration of the DF solution is 25 mM to 800 mM, and b. concentrating by ultrafiltration the oligonucleotide compound in the intermediate solution to obtain a high concentration, liquid composition, wherein the concentration of the oligonucleotide compound in the high concentration, liquid composition is greater than 150 mg/mL.

[0014] The present disclosure also provides a high concentration, liquid composition prepared by the method of the present disclosure. In various instances, the high concentration, liquid composition comprises the oligonucleotide compound at a concentration of about 150 mg/mL or greater, e.g., about 175 mg/mL, about 180 mg/mL, about 185 mg/mL, about 190 mg/mL, about 195 mg/mL, about 200 mg/mL, about 205 mg/mL, about 210 mg/mL, about 215 mg/mL, about 220 mg/mL, about 225 mg/mL, about 230 mg/mL, about 235 mg/mL, about 240 mg/mL, about 245 mg/mL, about 250 mg/mL, or greater. A frozen preparation made by storing the presently disclosed liquid composition at a temperature below 0 degrees C is additionally provided. The frozen preparation in exemplary examples is not a lyophilized or freeze-dried preparation.

[0014a] In another aspect, the present disclosure provides for a high concentration, liquid composition prepared by the method as disclosed herein. In yet another aspect, the present disclosure provides for a frozen preparation made by storing the liquid composition as disclosed herein at a temperature below 0 °C, optionally, at -20 °C.

[0015] The present disclosure further provides methods of manufacturing a medicament comprising an oligonucleotide compound. In exemplary examples, the method comprises carrying out the method of the present disclosure to obtain a high concentration, liquid composition comprising greater than about 150 mg/mL of the oligonucleotide compound, formulating the high concentration, liquid composition with a pharmaceutically acceptable excipient, and filling the formulated high concentration, liquid composition into a container.

[0015a] In a further aspect, the present disclosure provides for a method of manufacturing a medicament comprising an oligonucleotide compound, comprising carrying out the method as disclosed herein to obtain a high concentration, liquid composition comprising at least 150 mg/mL of the oligonucleotide compound, formulating the high concentration, liquid composition with a pharmaceutically-acceptable excipient, and filling the formulated high concentration, liquid composition into a container.

[0016] Methods of treating a subject with a disease are furthermore provided. In exemplary examples, the method comprises administering to the subject a liquid composition of the present disclosure, e.g., a liquid DP, in an amount effective to treat the disease in the subject. Optionally, the medicament is administered by injection or infusion.

[0017] Use of the high concentration, liquid composition of the present disclosure in the manufacture of a medicament for treating a disease in a subject is moreover provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Figure 1A is a flow diagram of a drug product manufacturing process with a powder drug substance. Figure 1B is a flow diagram of a drug product manufacturing process with a bulk liquid drug substance.

[0019] Figure 2 is a diagram of an exemplary method for preparing a high concentration, liquid composition comprising an oligonucleotide compound. The direction of movement of solutions is shown with solid black arrows.

[0020] Figure 3 depicts the structure of a model oligonucleotide compound schematically. The top strand listed in the 5' to 3' direction is the sense strand (SEQ ID NO: 3) and the bottom strand listed in the 3' to 5' direction is the antisense strand (SEQ ID NO: 4). Black circles

WHAT IS CLAIMED IS:

1. A method of preparing a high concentration, liquid composition comprising an oligonucleotide compound, said method comprising:
 - a. exchanging by diafiltration an oligonucleotide compound in a starting solution into a diafiltration (DF) solution to obtain an intermediate solution, wherein the concentration of the oligonucleotide compound in the starting solution is 140 mg/mL or less and the DF solution comprises one or more salts and the total salt concentration of the DF solution is 25 mM to 800 mM, and
 - b. concentrating by ultrafiltration the oligonucleotide compound in the intermediate solution to obtain a high concentration, liquid composition, wherein the concentration of the oligonucleotide compound in the high concentration, liquid composition is greater than 150 mg/mL.
2. The method of claim 1, wherein
 - a) the diafiltration is continuous diafiltration and/or the ultrafiltration is ultrafiltration by tangential flow filtration;
 - b) the method employs a polyethersulfone (PES) membrane or a stabilized cellulose membrane for the diafiltration and/or ultrafiltration; and/or
 - c) the membrane has a molecular weight cut-off (MWCO) of less than 10 kDa.
3. The method of claim 1 or 2, wherein the DF solution comprises, consists essentially of, or consists of an inorganic salt, optionally (i) wherein the inorganic salt comprises a monovalent cation, (ii) the inorganic salt comprises an alkali metal, optionally, sodium, potassium, or lithium, (iii) the inorganic salt comprises a halogen counterion, optionally chloride or bromide, and/or (iv) the inorganic salt is sodium chloride, sodium bromide, potassium chloride, or lithium chloride.
4. The method of claim 3, wherein the monovalent cation is an ammonium cation, optionally, wherein the inorganic salt is ammonium chloride or the inorganic salt comprises a divalent cation, optionally, wherein the divalent cation is an alkaline earth metal, optionally, magnesium or calcium.
5. The method of claim 4, wherein the inorganic salt is magnesium chloride or calcium chloride.

6. The method of any one of claims 1 to 5, wherein the DF solution comprises, consists essentially of, or consists of an organic salt and/or (b) the DF solution comprises a primary salt and at least one other salt.
7. The method of claim 6, wherein the organic salt comprises a quaternary ammonium cation, optionally, wherein the organic salt is choline chloride or benzyltrimethylammonium chloride.
8. The method of any one of the preceding claims, wherein
 - a) the total salt concentration of the DF solution is 25 mM to 500 mM, optionally, wherein:
 - i. the total salt concentration of the DF solution is 25 mM to 250 mM,
 - ii. the DF solution comprises, consists essentially of, or consists of an inorganic salt comprising a divalent cation, optionally, calcium chloride or magnesium chloride, and/or
 - iii. the total salt concentration of the DF solution is 75 mM to 250 mM, and/or
 - b) the total salt concentration of the DF solution per one negative charge of the oligonucleotide compound is 3.0 mM to 18.0 mM, optionally, wherein the total salt concentration of the DF solution per negative charge of the oligonucleotide compound is at least 3.0 mM or about 3.5 mM.
9. The method of claim 8, wherein the DF solution comprises, consists essentially of, or consists of an inorganic salt comprising a monovalent cation, optionally, wherein the DF solution comprises 75 mM to 300 mM sodium chloride, sodium bromide, lithium chloride, potassium chloride, or ammonium chloride.
10. The method of claim 6, wherein the concentration of the primary salt is at least 2-fold greater than the concentration of the other salt(s) of the DF solution, optionally, wherein the concentration of the primary salt is at least 3-fold greater than the concentration of the other salt(s) of the DF solution.
11. The method of any one of the preceding claims, wherein the DF solution comprises a buffer, optionally, wherein the buffer is phosphate buffered saline (PBS).
12. The method of any one of claims 6-11, wherein (a) the concentration of the primary salt in the DF solution is based on the concentration of the oligonucleotide compound present in the starting solution, (b) the molar ratio of the oligonucleotide compound of the starting solution to the primary salt of the DF solution is 1:2 to 1:100, 1:3 to 1:80, or 1:5 to 1:65, (c) the concentration of the primary salt of the DF solution is

at least 80% of the total salt concentration of the DF solution, optionally, the concentration of the primary salt of the DF solution is at least 90% of the total salt concentration of the DF solution, (d) the primary salt concentration of the DF solution is 50 mM to 500 mM, optionally, 100 mM to 600 mM, (e) the primary salt comprises a monovalent cation, optionally, sodium, (f) the primary salt comprises a divalent cation, optionally, magnesium or calcium.

13. The method of claim 12, wherein the primary salt is sodium chloride or magnesium chloride.
14. The method of any one of the preceding claims, wherein the DF solution is substantially free of potassium.
15. The method of any one of the preceding claims, wherein
 - a) the method further comprises finishing or polishing the final retentate, wherein the method, optionally, further comprises collecting the final retentate and filling it into a container with a total oligonucleotide recovery of greater than 80%;
 - b) the method does not comprise lyophilization of the oligonucleotide compound;
 - c) the oligonucleotide compound is double stranded or single stranded;
 - d) the oligonucleotide compound is an siRNA; and/or
 - e) the concentration of the oligonucleotide compound of the final retentate in the retentate vessel is at least 2-times higher than the oligonucleotide compound concentration of the starting solution.
16. A high concentration, liquid composition prepared by the method of any one of the preceding claims.
17. A frozen preparation made by storing the liquid composition of claim 16 at a temperature below 0 °C, optionally, at -20 °C.
18. A method of manufacturing a medicament comprising an oligonucleotide compound, comprising carrying out the method of any one of claims 1-15 to obtain a high concentration, liquid composition comprising at least 150 mg/mL of the oligonucleotide compound, formulating the high concentration, liquid composition with a pharmaceutically-acceptable excipient, and filling the formulated high concentration, liquid composition into a container.
19. Use of the high concentration, liquid composition of claim 16 in the manufacture of a medicament for treating a disease in a subject.