

formulations are not only difficult to handle during manufacturing, including at the bulk and filling stages, but they are also difficult to draw into a syringe and inject, making administration to the patient difficult and unpleasant.

To reduce viscosity of antibody formulations, unmodified arginine, glycine, serine, or proline amino acids have been added to antibody compositions. For example, antibody formulations containing 80 mg/mL of antibody and 75 mg/mL to about 125 mg/mL of arginine can be lyophilized and reconstituted to 120-200 mg/mL; the final arginine concentrations can be 431 mM to 718 mM (Morichika & Kameoka, 2007). While arginine reduced the viscosity of the formulations when compared to controls (Morichika & Kameoka, 2007). Furthermore, the effect of arginine was not sufficient to reduce evolocumab viscosity to desired levels.

There is a need in the art to reduce the viscosity of evolocumab- and other PCSK9-binding polypeptides-containing formulations with compounds that are more efficient than arginine.

SUMMARY

In one aspect, the present disclosure refers to a use of a pharmaceutical composition in the preparation of a medicament for treating ~~a cholesterol related disorder~~ familial hypercholesterolemia, non-familial hypercholesterolemia, hyperlipidemia, heart disease, metabolic syndrome, diabetes, coronary heart disease, stroke, cardiovascular disease, Alzheimer's disease or dyslipidemia in a human subject, wherein the pharmaceutical composition comprises:

- a. a PCSK9-binding polypeptide that selected from the group consisting of:
 - i. a monoclonal antibody comprising a heavy chain polypeptide having an amino acid sequence of SEQ ID NO:1 and a light chain polypeptide having an amino acid sequence of SEQ ID NO:2 (evolocumab), or an antigen-binding fragment thereof; and
 - ii. a monoclonal antibody, comprising:

NOs:14; a heavy chain CDR2 that is a CDR2 in SEQ ID NOs:14; a heavy chain CDR3 that is a CDR3 in SEQ ID NOs:14, and

- ii. a light chain polypeptide comprising the following CDRs: a light chain CDR1 that is a CDR1 in SEQ ID NOs:15; a light chain CDR2 that is a CDR2 in SEQ ID NOs:15; and a light chain CDR3 that is a CDR3 in SEQ ID NOs:15,

wherein the pharmaceutical composition has a viscosity of less than 80 cP.

In yet another aspect one example, the present disclosure refers to a method of formulating a therapeutic polypeptide, comprising

- a. a first concentration step, wherein the polypeptide in a first solution is concentrated;
- b. a first solution exchange step, wherein the concentrated polypeptide in the first solution is exchanged into a second solution comprising N-acetyl arginine using diafiltration;
- c. a second concentration step, wherein the polypeptide in the second solution is concentrated;
- d. a second solution exchange step, wherein the polypeptide in the concentrated second solution is exchanged into a third solution comprising N-acetyl arginine using diafiltration; and
- e. a third concentration step, wherein the polypeptide in the third solution is concentrated;

wherein the therapeutic polypeptide comprises a PCSK9-binding polypeptide that blocks binding

of PCSK9 to LDLR and is selected from the group consisting of:

- i. a monoclonal antibody comprising a heavy chain having an amino acid sequence of SEQ ID NO:1 and a light chain having an amino acid sequence of SEQ ID NO:2 (evolocumab), or an antigen-binding fragment thereof; and
- ii. a monoclonal antibody, comprising:

v. a monoclonal antibody that binds to PCSK9 at an epitope on PCSK9 that overlaps with an epitope that is bound by an antibody that comprises:

1. a heavy chain variable region of the amino acid sequence in SEQ ID NO:1; and

2. a light chain variable region of the amino acid sequence in SEQ ID NO:2, and

3. wherein the epitope of the monoclonal antibody further overlaps with a site to which an EGF-A domain of LDLR;

b. N-acetyl arginine present at a concentration of about 155 mM;

c. proline present at a concentration of about 120 mM;

d. polyoxyethylenesorbitan monooleate (polysorbate 80) at a concentration of about 0.005% (w/v) to about 0.015% (w/v); and

e. sodium acetate at a concentration of about 10 mM.

In this example, the pharmaceutical composition can have a pH of about 5.1 to about 5.7, such as a pH of about 5.4. The pharmaceutical composition can have a viscosity of at least less than about 60 cP.

In these examples, the PCSK9-binding polypeptide can be stable for at least about two years or even five years or more when stored at about -30 °C or colder in the pharmaceutical compositions as described. At 5 °C, the PCSK9-binding polypeptide can be stable for at least about six months to about 24 months or more in such pharmaceutical compositions. At 25 °C, the PCSK9-binding polypeptide can be stable for at least about one month or longer, three months or longer, or even six months or longer. At 40 °C, the PCSK9-binding polypeptide can be stable for at least one month or longer. The pharmaceutical compositions as described can comprise high molecular weight aggregates or oligomers of PCSK9-binding polypeptides at less than about 3%, such as 2.5% or less, of the total PCSK9-binding polypeptide concentration.

In any of the preceding examples, the pharmaceutical composition can be a liquid.

In another example, disclosed herein is a method of treating a subject in need thereof, comprising administering the pharmaceutical composition of any of the preceding examples.

In yet another aspect, the present disclosure refers to the use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a cholesterol related disorder in a subject in need thereof.

In another ~~aspect~~example, the present disclosure refers to a kit comprising a pharmaceutical composition as disclosed herein and a delivery device. The delivery device can be selected from the group consisting of a syringe, an injector pen, a body injector, and an autoinjector. The kit can further comprise instructions for administering the pharmaceutical composition using the delivery device.

5 In another example, disclosed herein is a method of preparing a PCSK9-binding polypeptide pharmaceutical composition comprising at least 140 mg/mL of PCSK9-binding polypeptide, comprising adding to a pharmaceutical composition comprising the PCSK9-binding polypeptide an effective amount of N-acetyl arginine, such that the viscosity of the pharmaceutical composition is reduced when compared to the pharmaceutical composition lacking the N-acetyl arginine, and wherein the PCSK9-
10 binding polypeptide is selected from the group consisting of:

a. a monoclonal antibody comprising a heavy chain polypeptide having an amino acid sequence of SEQ ID NO:1 and a light chain polypeptide having an amino acid sequence of SEQ ID NO:2 (evolocumab), or an antigen-binding fragment thereof;

b. a monoclonal antibody that competes with evolocumab for binding to PCSK9;

15 c. a monoclonal antibody, comprising:

i. a heavy chain polypeptide comprising the following complementarity determining regions (CDRs): a heavy chain CDR1 that is a CDR1 in SEQ ID NOs:14 or 16; a heavy chain CDR2 that is a CDR2 in SEQ ID NOs:14 or 16; a heavy chain CDR3 that is a CDR3 in SEQ ID NOs:14 or 16, and

20 ii. a light chain polypeptide comprising the following CDRs: a light chain CDR1 that is a CDR1 in SEQ ID NOs:15 or 17; a light chain CDR2 that is a CDR2 in SEQ ID NOs:15 or 17; and a light chain CDR3 that is a CDR3 in SEQ ID NOs:15 or 17;

d. a monoclonal antibody that binds to at least one of the following residues of PCSK9, the PCSK9 comprising an amino acid sequence of SEQ ID NO:3: S153, D188, I189, Q190, S191, D192, R194, E197, G198, R199, V200, D224, R237, and D238, K243, S373, D374, S376, T377, F379, I154, T1897, H193, E195, I196, M201, V202, C223, T228, S235, G236, A239, G244, M247, I369, S372, C375, C378, R237, and D238; and

e. a monoclonal antibody that binds to PCSK9 at an epitope on PCSK9 that overlaps with an epitope that is bound by an antibody that comprises:

30 i. a heavy chain variable region of the amino acid sequence in SEQ ID NO:1; and

ii. a light chain variable region of the amino acid sequence in SEQ ID NO:2, and

CLAIMS

1. Use of a pharmaceutical composition in the preparation of a medicament for treating familial hypercholesterolemia, non-familial hypercholesterolemia, hyperlipidemia, heart disease, metabolic syndrome, diabetes, coronary heart disease, stroke, cardiovascular disease, Alzheimer's disease or dyslipidemia ~~a cholesterol-related disorder~~ in a human subject, wherein the pharmaceutical composition comprises:
 - a. a PCSK9-binding polypeptide that selected from the group consisting of:
 - i. a monoclonal antibody comprising a heavy chain polypeptide having an amino acid sequence of SEQ ID NO:1 and a light chain polypeptide having an amino acid sequence of SEQ ID NO:2 (evolocumab), or an antigen-binding fragment thereof; and
 - ii. a monoclonal antibody, comprising:
 1. a heavy chain polypeptide comprising the following complementarity determining regions (CDRs): a heavy chain CDR1 that is a CDR1 in SEQ ID NOs:14; a heavy chain CDR2 that is a CDR2 in SEQ ID NOs:14; a heavy chain CDR3 that is a CDR3 in SEQ ID NOs:14, and
 2. a light chain polypeptide comprising the following CDRs: a light chain CDR1 that is a CDR1 in SEQ ID NOs:15; a light chain CDR2 that a CDR2 in SEQ ID NOs:15; and a light chain CDR3 that is a CDR3 in SEQ ID NOs:15;
 - and
 - b. N-acetyl arginine, and
 - c. at least one of an arginine salt or proline,
 - wherein the pharmaceutical composition has a viscosity of less than 80 cP.
2. The use of claim 1, wherein the PCSK9-binding polypeptide is a monoclonal antibody thate comprises:
 - a heavy chain polypeptide comprising the following complementarity determining regions (CDRs): heavy chain CDR1, CDR2, and CDR3 having an amino acid sequence of SEQ ID NOs:7, 8, and 9, respectively; and
 - a light chain polypeptide comprising the following CDRs: light chain CDR1, CDR2, and CDR3 having an amino acid sequence of SEQ ID NOs:4, 5, and 6, respectively.
3. The use of claim 1, wherein the PCSK9-binding polypeptide is present at a concentration of 140 mg/mL to 260 mg/mL.

4. The use of claim 1 or 2, wherein the PCSK9-binding polypeptide concentration is 210 mg/mL.
5. The use of any of claims 1-4, wherein the N-acetyl arginine is present at a concentration from 25 mM to 230 mM.
6. The use of any of claims 1-5, wherein the pharmaceutical composition further comprises a buffer.
7. The use of any of claim 1-6, wherein the pharmaceutical composition further comprises a surfactant.
8. The use of any of claims 1-7, wherein the pharmaceutical composition further comprises proline.
9. The use of any of claims 1-8, wherein the pharmaceutical composition further comprises the arginine salt.
10. The use of any of claims 1-9, wherein the composition comprises high molecular weight aggregates or oligomers of the PCSK9-binding polypeptide at less than 3% of the PCSK9-binding polypeptide concentration.
11. The use of any of claims 1-11, wherein the pharmaceutical composition is liquid.
12. The use of any of claims 1-11, wherein the medicament is comprised in a kit, wherein the kit further comprises a delivery device selected from the group consisting of a syringe, an injector pen, a body injector, and an autoinjector.
13. The use of any one of claims 1-12, wherein the familial hypercholesterolemia is heterozygous hypercholesterolemia, homozygous hypercholesterolemia, familial defective apop lipoprotein B-100; polygenic hypercholesterolemia; remnant removal disease, or hepatic lipase deficiency.
14. The use of any one of claims 1-12, wherein the dyslipidemia is diabetes mellitus, familial combined hyperlipidemia, familial hypertriglyceridemia, or dyslipidemia secondary to one of: dietary indiscretion, hypothyroidism, drugs including estrogen and progestin therapy, beta-blockers, and thiazide diuretics; nephrotic syndrome, chronic renal failure, Cushing's syndrome, primary biliary cirrhosis, glycogen storage diseases, hepatoma, cholestasis, acromegaly, insulinoma, isolated growth hormone deficiency, and alcohol-induced hypertriglyceridemia.
- ~~13,15.~~ 15. A method of preparing a PCSK9-binding polypeptide in a pharmaceutical composition comprising at least 140 mg/mL of PCSK9-binding polypeptide, comprising adding to a pharmaceutical composition comprising the PCSK9-binding polypeptide an effective amount of N-acetyl arginine, such that the viscosity of the pharmaceutical composition is reduced when

compared to the pharmaceutical composition lacking the N-acetyl arginine, and wherein the PCSK9-binding polypeptide is selected from the group consisting of:

- a. a monoclonal antibody comprising a heavy chain polypeptide having an amino acid sequence of SEQ ID NO:1 and a light chain polypeptide having an amino acid sequence of SEQ ID NO:2 (evolocumab), or an antigen-binding fragment thereof; and
- b. a monoclonal antibody, comprising:
 - i. a heavy chain polypeptide comprising the following complementarity determining regions (CDRs): a heavy chain CDR1 that is a CDR1 in SEQ ID NOs:14; a heavy chain CDR2 that is a CDR2 in SEQ ID NOs:14; a heavy chain CDR3 that is a CDR3 in SEQ ID NOs:14, and
 - ii. a light chain polypeptide comprising the following CDRs: a light chain CDR1 that is a CDR1 in SEQ ID NOs:15; a light chain CDR2 that is a CDR2 in SEQ ID NOs:15; and a light chain CDR3 that is a CDR3 in SEQ ID NOs:15,

wherein the pharmaceutical composition has a viscosity of less than 80 cP.

~~14. A method of formulating a therapeutic polypeptide, comprising~~

- ~~a. a first concentration step, wherein the polypeptide in a first solution is concentrated;~~
- ~~b. a first solution exchange step, wherein the concentrated polypeptide in the first solution is exchanged into a second solution comprising N-acetyl arginine using diafiltration;~~
- ~~c. a second concentration step, wherein the polypeptide in the second solution is concentrated;~~
- ~~d. a second solution exchange step, wherein the polypeptide in the concentrated second solution is exchanged into a third solution comprising N-acetyl arginine using diafiltration; and~~
- ~~e. a third concentration step, wherein the polypeptide in the third solution is concentrated;~~

~~wherein the therapeutic polypeptide comprises a PCSK9-binding polypeptide that blocks binding of PCSK9 to LDLR and is selected from the group consisting of:~~

- ~~i. a monoclonal antibody comprising a heavy chain having an amino acid sequence of SEQ ID NO:1 and a light chain having an amino acid sequence of SEQ ID NO:2 (evolocumab), or an antigen-binding fragment thereof; and~~
- ~~ii. a monoclonal antibody, comprising:~~

1. ~~a heavy chain polypeptide comprising the following complementarity determining regions (CDRs): a heavy chain CDR1 that is a CDR1 in SEQ ID NOs:14; a heavy chain CDR2 that is a CDR2 in SEQ ID NOs:14; a heavy chain CDR3 that is a CDR3 in SEQ ID NOs:14, and~~
 2. ~~a light chain polypeptide comprising the following CDRs: a light chain CDR1 that is a CDR1 in SEQ ID NOs:15; a light chain CDR2 that is a CDR2 in SEQ ID NOs:15; and a light chain CDR3 that is a CDR3 in SEQ ID NOs:15.~~
15. The method of claim 14.e.ii, wherein the PCSK9 binding polypeptide that blocks binding of PCSK9 to LDLR is a monoclonal antibody the comprises:
- a heavy chain polypeptide comprising the following complementarity determining regions (CDRs):
- heavy chain CDR1, CDR2, and CDR3 having an amino acid sequence of SEQ ID NOs: 7, 8, and 9, respectively; and
- a light chain polypeptide comprising the following CDRs: light chain CDR1, CDR2, and CDR3 having an amino acid sequence of SEQ ID NOs: 4, 5, and 6, respectively.
16. The method of claim 14.e.ii, wherein before the third concentration step, the temperature of the solution comprising the polypeptide is increased from 25 °C to 37 °C.
17. The method of claim 14.e.ii, wherein in the first concentration step, the therapeutic polypeptide concentration is increased from 3 to 7 fold.
18. The method of claim 14.e.ii, wherein in the second concentration step, the therapeutic polypeptide concentration is increased from 2 to 4 fold from the first concentration step.
19. The method of claim 14.e.ii, wherein in the third concentration step, the therapeutic polypeptide concentration is increased from 1.5 to 2 fold from the second concentration step.
20. The method of claim 14.e.ii, wherein the therapeutic polypeptide has a final concentration that is at least 19-20 fold more concentrated than the initial concentration of the therapeutic polypeptide.
21. The method of claim 14.e.ii, wherein the concentration steps comprise fed batch ultrafiltration.
22. The method of any of claims 15-22, wherein the second or third solution has a pH from 4.8 to 6.9.
23. The method of claim 14.e.ii, wherein in the first and second solution exchange steps, a diafiltration membrane is used having at least one characteristic selected from the group consisting of:

- a. ~~mesh openings that are greater than 350 μm but less than or equal to 500 μm ;~~
- b. ~~an open area that is greater than 32% but less than or equal to 36% of the membrane area;~~
- c. ~~a mesh count of less than 16.2 n/cm but greater than or equal to 12.2 n/cm;~~
- d. ~~a wire diameter that is greater than 270 μm but less than or equal to 340 μm ;~~
- e. ~~a basis weight that is greater than 160 g/m² but less than or equal to 180 g/m²;~~
- f. ~~a thickness greater than 515 μm but less than or equal to 610 μm ;~~
- g. ~~a membrane load of greater than 1138.1 g/m² but less than or equal to 1919.3 g/m²;~~
and
- h. ~~a maximum feed pressure of 60 psi.~~

24. ~~The method of any of claims 14.e.ii to 23, wherein surfactant is added to the third solution after being concentrated.~~