

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 pharmaceutical composition comprising

- 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;
 - ii. a monoclonal antibody that competes with evolocumab for binding to PCSK9;
 - iii. 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;
 - iv. 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, T187, H193, E195, I196, M201, V202, C223, T228, S235, G236, A239, G244, M247, I369, S372, C375, C378, R237, and D238;

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

b. N-acetyl arginine,

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

In another aspect, the present disclosure refers to 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;

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

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; 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;

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, T187, 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:
- a heavy chain variable region of the amino acid sequence in SEQ ID NO:1; and
 - a light chain variable region of the amino acid sequence in SEQ ID NO:2.

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

- a first concentration step, wherein the polypeptide in a first solution is concentrated;
- 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;
- a second concentration step, wherein the polypeptide in the second solution is concentrated;
- 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
- 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:

- 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;
- a monoclonal antibody that competes with evolocumab for binding to PCSK9;
- a monoclonal antibody, comprising:
 - 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;
- iv. 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, D238, K243, S373, D374, S376, T377, F379, I154, T187, H193, E195, I196, M201, V202, C223, T228, S235, G236, A239, G244, M247, I369, S372, C375, C378, R237, D238;
- 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.

In an example, provided herein is a pharmaceutical composition comprising

- 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;
 - ii. a monoclonal antibody that competes with evolocumab for binding to PCSK9;
 - iii. 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 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
 2. 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;
 - iv. 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;

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;

and

b. N-acetyl arginine,

wherein the pharmaceutical composition has a viscosity of at least less than about 80 cP. In such an example, the PCSK9-binding polypeptide can be a monoclonal antibody that comprises a heavy chain polypeptide comprising the following complementarity determining regions (CDRs):

a. heavy chain CDR1, CDR2, and CDR3 having an amino acid sequence of SEQ ID NOs:7, 8, and 9, respectively; and

b. light chain CDR1, CDR2, and CDR3 having an amino acid sequence of SEQ ID NOs:4, 5, and 6, respectively. Furthermore, in this example, the pharmaceutical composition can have a viscosity of at least less than about 50 cP. The pharmaceutical composition can have an osmolality of about 250 to about 400 mOsm/kg, such as about 300 mOsm/kg, or is isotonic to a human blood cell. The concentration of the PCSK9-binding polypeptide can be from about 140 mg/mL to about 260 mg/mL, such as 210 mg/mL. The N-acetyl arginine can be present at a concentration from about 25 mM to about 230 mM, such as 140 mM to about 170 mM, or 140 mM. The pharmaceutical composition as described can further comprise a buffer, such as a buffer selected from the group consisting of acetate, glutamate, histidine, and phosphate buffers, or a combination thereof. The buffer can be present at a concentration from about 5 mM to about 30 mM. In some cases, the buffer is sodium acetate and is present at a concentration of about 10 mM. The pH of such pharmaceutical compositions can be from about 4.8 to about 6.9, such as a pH of about 5.4. The pharmaceutical compositions as described can further comprise a surfactant, such as a surfactant selected from the group consisting of polyoxyethylenesorbitan monooleate (polysorbate 80 or polysorbate 20), polyoxyethylene-polyoxypropylene block copolymer (Poloxamers such as Pluronic® F-68 and other Pluronics®), Sorbitan

alkyl esters (Spans®) Polyethylene glycol octylphenyl ethers (Triton X-100), Polyethylene glycol alkyl ethers (Brij), Polypropylene glycol alkyl ethers, Glucoside alkyl ethers, and D-α-tocopherol polyethylene glycol succinate (vitamin E TPGS). The surfactant can be present at a concentration of about 0.0001% (w/v) to about 1% (w/v). In some pharmaceutical compositions as described, the surfactant is polyoxyethylenesorbitan monooleate (polysorbate 80) and is present at a concentration of about 0.01% (w/v). Furthermore, the pharmaceutical compositions as described can further comprise proline, which can be present at a concentration of about 50 mM to about 150 mM, such as 90 to 120 mM, or about 120 mM. In some cases, the pharmaceutical composition as described can further comprise an arginine salt, which can be present at a concentration of about 25 mM to about 150 mM, such as about 50 mM to about 100 mM. The arginine salt can be, for example, arginine-HCl, arginine acetate, or arginine glutamate. In some cases, the arginine salt is arginine HCl and is present at a concentration of about 50 mM. 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 an example, disclosed herein is a pharmaceutical composition comprising

- 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;
 - ii. a monoclonal antibody that competes with evolocumab for binding to PCSK9;
 - iii. 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 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

2. 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;

iv. 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;

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;

and

b. N-acetyl arginine;

c. an arginine salt;

d. a buffer; and

e. a surfactant

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

In another example, the PCSK9-binding polypeptide is a monoclonal antibody that comprises a heavy chain polypeptide comprising the following complementarity determining regions (CDRs):

a. heavy chain CDR1, CDR2, and CDR3 having an amino acid sequence of SEQ ID NOs:7, 8, and 9, respectively; and

b. light chain CDR1, CDR2, and CDR3 having an amino acid sequence of SEQ ID NOs:4, 5, and 6, respectively.

The pharmaceutical compositions as described can have a viscosity of at least less than about 50 cP. The pharmaceutical composition can have an osmolality of about 250 to about 400 mOsm/kg, such as about 300 mOsm/kg, or is isotonic to a human blood cell. The concentration of the PCSK9-binding polypeptide can be from about 140 mg/mL to about 260 mg/mL, such as 210 mg/mL. The N-acetyl

arginine can be present at a concentration from about 25 mM to about 230 mM, such as 140 mM to about 170 mM, or 140 mM. The pharmaceutical composition as described can further comprise a buffer, such as a buffer selected from the group consisting of acetate, glutamate, histidine, and phosphate buffers, or a combination thereof. The buffer can be present at a concentration from about 5 mM to about 30 mM. In some cases, the buffer is sodium acetate and is present at a concentration of about 10 mM. The pH of such pharmaceutical compositions can be from about 4.8 to about 6.9, such as a pH of about 5.4. The pharmaceutical compositions as described can further comprise a surfactant, such as a surfactant selected from the group consisting of polyoxyethylenesorbitan monooleate (polysorbate 80 or polysorbate 20), polyoxyethylene-polyoxypropylene block copolymer (Poloxamers such as Pluronic® F-68 and other Pluronics®), Sorbitan alkyl esters (Spans®) Polyethylene glycol octylphenyl ethers (Triton X-100), Polyethylene glycol alkyl ethers (Brij), Polypropylene glycol alkyl ethers, Glucoside alkyl ethers, and D-α-tocopherol polyethylene glycol succinate (vitamin E TPGS). The surfactant can be present at a concentration of about 0.0001% (w/v) to about 1% (w/v). In some pharmaceutical compositions as described, the surfactant is polyoxyethylenesorbitan monooleate (polysorbate 80) and is present at a concentration of about 0.01% (w/v). Furthermore, the pharmaceutical compositions as described can further comprise proline; the proline can be present at a concentration of about 50 mM to about 150 mM, such as 90 to 120 mM, or about 120 mM. In some cases, the pharmaceutical composition as described can further comprise an arginine salt, which can be present at a concentration of about 25 mM to about 150 mM, such as about 50 mM to about 100 mM. The arginine salt can be, for example, arginine-HCl, arginine acetate, or arginine glutamate. In some cases, the arginine salt is arginine HCl and is present at a concentration of about 50 mM. 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 of this example. 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 another example, disclosed herein is a pharmaceutical composition comprising

- a. a PCSK9-binding polypeptide that at a concentration of about 195-225 mg/mL 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;

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

iii. 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 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

2. 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;

iv. 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;

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 140 mM;

c. arginine HCl present at a concentration of about 50 mM;

d. polyoxyethylenesorbitan monooleate (polysorbate 80) at a concentration of from 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 50 cP.

In another example, disclosed herein is a pharmaceutical composition comprising

a. a PCSK9-binding polypeptide that at a concentration of about 195-225 mg/mL 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;

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

iii. 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 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

2. 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;

iv. 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;

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

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

c. N-acetyl arginine present at a concentration of about 140 mM;

d. arginine HCl present at a concentration of about 63 mM;

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

f. 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 80 cP.

In another example, disclosed herein is a pharmaceutical composition comprising

5 a. a PCSK9-binding polypeptide that at a concentration of about 195-225 mg/mL 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;

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

iii. 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 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

15 2. 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;

20 iv. 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;

25 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

30 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. arginine HCl present at a concentration of about 70 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.

5 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 45 cP.

In another example, disclosed herein is a pharmaceutical composition comprising

- a. a PCSK9-binding polypeptide that at a concentration of about 195-225 mg/mL selected

10 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;

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

- 15 iii. 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 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 2. 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;

- iv. 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;

- 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:

- 30 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;

5 b. N-acetyl arginine present at a concentration of about 170 mM;

c. arginine HCl present at a concentration of about 63 mM;

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

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

10 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 another example, disclosed herein is a pharmaceutical composition comprising

15 a. a PCSK9-binding polypeptide that at a concentration of about 195-225 mg/mL 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;

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

iii. 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 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

25 2. 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;

30 iv. 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;

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 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, 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

25 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

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

In this example, the viscosity of the pharmaceutical composition is less than about 80 cP, or less than about 50 cP. The pharmaceutical composition of method as disclosed can have an osmolality of about 250 to about 400 mOsm/kg, such as about 300 mOsm/kg, or is isotonic to a human blood cell. The concentration of the PCSK9-binding polypeptide can be from about 140 mg/mL to about 260 mg/mL, such as 210 mg/mL. The N-acetyl arginine can be present at a concentration from about 25 mM to about 230 mM, such as 140 mM to about 170 mM, or 140 mM. The pharmaceutical composition as described can further comprise a buffer, such as a buffer selected from the group consisting of acetate, glutamate, histidine, and phosphate buffers, or a combination thereof. The buffer can be present at a concentration from about 5 mM to about 30 mM. In some cases, the buffer is sodium acetate and is present at a concentration of about 10 mM. The pH of such pharmaceutical compositions can be from about 4.8 to about 6.9, such as a pH of about 5.4. The pharmaceutical compositions as described can further comprise a surfactant, such as a surfactant selected from the group consisting of polyoxyethylenesorbitan monooleate (polysorbate 80 or polysorbate 20), polyoxyethylene-polyoxypropylene block copolymer (Poloxamers such as Pluronic® F-68 and other Pluronics®), Sorbitan alkyl esters (Spans®) Polyethylene glycol octylphenyl ethers (Triton X-100), Polyethylene glycol alkyl ethers (Brij), Polypropylene glycol alkyl ethers, Glucoside alkyl ethers, and D-α-tocopherol polyethylene glycol succinate (vitamin E TPGS). The surfactant can be present at a concentration of about 0.0001% (w/v) to about 1% (w/v). In some pharmaceutical compositions as described, the surfactant is polyoxyethylenesorbitan monooleate (polysorbate 80) and is present at a concentration of about 0.01% (w/v). Furthermore, the pharmaceutical compositions as described can further comprise proline; the proline can be present at a concentration of about 50 mM to about 150 mM, such as 90 to 120 mM, or about 120 mM. In some cases, the pharmaceutical composition as described can further comprise an arginine salt, which can be present at a concentration of about 25 mM to about 150 mM, such as about 50 mM to about 100 mM. The arginine salt can be, for example, arginine-HCl, arginine acetate, or arginine glutamate. In some cases, the arginine salt is arginine HCl and is present at a concentration of about 50 mM. 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.

5 In another example, disclosed herein is 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;
- 10 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
- 15 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:

- 20 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;
- ii. a monoclonal antibody that competes with evolocumab for binding to PCSK9;
- iii. a monoclonal antibody, comprising:
 - 25 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 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
 - 30 2. 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;

- iv. 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, 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, D238;
- 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 EGFA domain of LDLR.

In the methods of this example, the PCSK9-binding polypeptide that blocks binding of PCSK9 to LDLR can be a monoclonal antibody that comprises a heavy chain polypeptide comprising the following complementarity determining regions (CDRs):

- a. heavy chain CDR1, CDR2, and CDR3 having an amino acid sequence of SEQ ID NOs: 7, 8, and 9, respectively; and
- b. light chain CDR1, CDR2, and CDR3 having an amino acid sequence of SEQ ID NOs: 4, 5, and 6, respectively.

Furthermore, in this example, before the third concentration step, the temperature of the solution comprising the polypeptide can be increased from about 25 °C to about 37 °C. Also, the first solution exchange step can be accomplished using at least three diavolumes of the second solution. In some examples as described, the second solution exchange step is accomplished using at least four diavolumes of the third solution. In other examples, the initial concentration of the therapeutic protein is about 11 mg/mL or less. Additionally, the therapeutic polypeptide concentration can be increased from about 3- to about 7-fold, such as, for example, where the increased concentration of the polypeptide is from about 35 mg/mL to about 70 mg/mL. In some examples, in the second concentration step, the therapeutic polypeptide concentration is increased from about 2- to 4-fold from the first concentration step, such as to about 140 mg/mL, for example. In the third concentration step,

the therapeutic polypeptide concentration can be increased from about 1.5- to about 2-fold from the second concentration step, such as to about 260 mg/mL. The therapeutic polypeptide can therefore have a final concentration that is at least about 19-20-fold more concentrated than the initial concentration of the therapeutic polypeptide, such as about 210 mg/mL. The concentration steps can
5 comprise fed-batch ultrafiltration; furthermore, the second solution and the third solution can be identical. For example, the second or third solution comprising N-acetyl arginine can comprise an arginine salt and a buffer, wherein, for example, the N-acetyl arginine is present at a concentration of about 25 mM to about 230 mM; the arginine salt is Arg HCl, Arg acetate, or Arg glutamate and is present at a concentration of about 25 mM to about 150 mM; and the buffer is a sodium acetate buffer at a
10 concentration of about 5 mM to about 30 mM. In other examples, the N-acetyl arginine is present at a concentration of about 140 to about 170 mM; the Arg HCl, Arg acetate, or Arg glutamate is present at a concentration of about 63 to about 70 mM and the sodium acetate buffer is present at a concentration of about 10 mM. In yet other examples, the N-acetyl arginine is present at a concentration of about 140 mM, the Arg HCl, Arg acetate, or Arg glutamate is present at a concentration of about 63 mM, the
15 sodium acetate buffer is present at a concentration of about 10 mM. In further examples, the N-acetyl arginine is present at a concentration of about 155 mM, the Arg HCl, Arg acetate, or Arg glutamate is present at a concentration of about 70 mM, the sodium acetate buffer is present at a concentration of about 10 mM. In yet other examples, the N-acetyl arginine is present at a concentration of about 170 mM, the Arg HCl, Arg acetate, or Arg glutamate is present at a concentration of about 63 mM, the
20 sodium acetate buffer is present at a concentration of about 10 mM. Furthermore, the compositions can further comprise proline, wherein the proline is present at a concentration of about 50 mM to about 150 mM. The second or third solution can have a pH from about 4.8 to about 6.9, such as 5.4. In the first and second solution exchange steps, a diafiltration membrane can be used having at least one characteristic selected from the group consisting of:

- 25 a. mesh openings that are greater than about 350 μm but less than or equal to about 500 μm ;
- b. an open area that is greater than about 32% but less than or equal to about 36% of the membrane area;
- c. a mesh count of less than about 16.2 n/cm but greater than or equal to about 12.2 n/cm;
- d. a wire diameter that is greater than about 270 μm but less than or equal to about 340 μm ;
- 30 e. a basis weight that is greater than about 160 g/m^2 but less than or equal to 180 g/m^2 ;
- f. a thickness greater than about 515 μm but less than or equal to about 610 μm ;

g. a membrane load of greater than about 1138.1 g/m² but less than or equal to about 1919.3 g/m²; and

h. a maximum feed pressure of about 60 psi.

Furthermore, surfactant can added to the third solution after being concentrated, such as a surfactant selected from the group consisting of polyoxyethylenesorbitan monooleate (polysorbate 80 or polysorbate 20), polyoxyethylene-polyoxypropylene block copolymer (Poloxamers such as Pluronic® F-68 and other Pluronics®), Sorbitan alkyl esters (Spans®) Polyethylene glycol octylphenyl ethers (Triton X-100), Polyethylene glycol alkyl ethers (Brij), Polypropylene glycol alkyl ethers, Glucoside alkyl ethers, and D-α-tocopherol polyethylene glycol succinate (vitamin E TPGS). The surfactant can be present at a concentration of about 0.0001% (w/v) to about 1% (w/v). In some pharmaceutical compositions as described, the surfactant is polyoxyethylenesorbitan monooleate (polysorbate 80) and is present at a concentration of about 0.01% (w/v).

In another example, disclosed herein is a method of formulating a therapeutic polypeptide, comprising

- a. a first concentration step, wherein the polypeptide in a first solution is concentrated using fed-batch ultrafiltration;
- b. a first solution exchange step, wherein the concentrated polypeptide in the first solution is exchanged into a second solution comprising N-acetyl arginine, arginine salt, and a buffer, using diafiltration and three diavolumes of the second solution;
- c. a second concentration step, wherein the polypeptide in the second solution is concentrated using fed-batch ultrafiltration;
- d. a second solution exchange step, wherein the polypeptide in the concentrated second solution is exchanged into third solution comprising N-acetyl arginine, arginine salt, and a buffer using diafiltration and four diavolumes of the third solution;
- e. the temperature of the solution comprising the polypeptide is increased from about 25 °C to about 37 °C after the second solution exchange step; and
- f. a third concentration step, wherein the polypeptide is further concentrated using fed-batch ultrafiltration concentration;

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 about 350 μm but less than or equal to about 500 μm;

- b. an open area that is greater than about 32% but less than or equal to about 36% of the membrane area;
- c. a mesh count of less than about 16.2 n/cm but greater than or equal to about 12.2 n/cm;
- d. a wire diameter that is greater than about 270 μm but less than or equal to about 340 μm ;
- e. a basis weight that is greater than about 160 g/m^2 but less than or equal to 180 g/m^2 ;
- f. a thickness greater than about 515 μm but less than or equal to about 610 μm ;
- g. a membrane load of greater than about 1138.1 g/m^2 but less than or equal to about 1919.3 g/m^2 ; and
- h. a maximum feed pressure of about 60 psi;

and

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;
- ii. a monoclonal antibody that competes with evolocumab for binding to PCSK9;
- iii. 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 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
 - 2. 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;
- iv. 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, 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, D238;

- 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 EGFA domain of LDLR.

Furthermore, in an example, the first solution exchange step can be accomplished using at least three diavolumes of the second solution. In some examples, the second solution exchange step is accomplished using at least four diavolumes of the third solution. In other examples, the initial concentration of the therapeutic protein is about 11 mg/mL or less. Additionally, the therapeutic polypeptide concentration can be increased from about 3- to about 7-fold, such as, for example, where the increased concentration of the polypeptide is from about 35 mg/mL to about 70 mg/mL. In some examples, in the second concentration step, the therapeutic polypeptide concentration is increased from about 2- to 4-fold from the first concentration step, such as to about 140 mg/mL, for example. In the third concentration step, the therapeutic polypeptide concentration can be increased from about 1.5- to about 2-fold from the second concentration step, such as to about 260 mg/mL. The therapeutic polypeptide can therefore have a final concentration that is at least about 19-20-fold more concentrated than the initial concentration of the therapeutic polypeptide, such as about 210 mg/mL. The concentration steps can comprise fed-batch ultrafiltration; furthermore, the second solution and the third solution can be identical. The N-acetyl arginine present at a concentration of about 25 mM to about 230 mM; the arginine salt can be Arg HCl, Arg acetate, or Arg glutamate, wherein the Arg HCl, Arg acetate, or Arg glutamate is present at a concentration of about 25 mM to about 150 mM; and the buffer is a sodium acetate buffer at a concentration of about 5 mM to about 30 mM. In other examples, the N-acetyl arginine is present at a concentration of about 140 to about 170 mM; the Arg HCl, Arg acetate, or Arg glutamate is present at a concentration of about 63 to about 70 mM and the sodium acetate buffer is present at a concentration of about 10 mM. In yet other examples, the N-acetyl arginine is present at a concentration of about 140 mM, the Arg HCl, Arg acetate, or Arg glutamate is present at a concentration of about 63 mM, the sodium acetate buffer is present at a concentration of about 10 mM. In further examples, the N-acetyl arginine is present at a concentration of about 155 mM, the Arg HCl, Arg acetate, or Arg glutamate is present at a concentration of about 70 mM, the sodium

acetate buffer is present at a concentration of about 10 mM. In yet other examples, the N-acetyl arginine is present at a concentration of about 170 mM, the Arg HCl, Arg acetate, or Arg glutamate is present at a concentration of about 63 mM, the sodium acetate buffer is present at a concentration of about 10 mM. Furthermore, the compositions can further comprise proline, wherein the proline is present at a concentration of about 50 mM to about 150 mM. The second or third solution can have a pH from about 4.8 to about 6.9, such as 5.4. Furthermore, surfactant can added to the third solution after being concentrated, such as a surfactant selected from the group consisting of polyoxyethylenesorbitan monooleate (polysorbate 80 or polysorbate 20), polyoxyethylene-polyoxypropylene block copolymer (Poloxamers such as Pluronic® F-68 and other Pluronics®), Sorbitan alkyl esters (Spans®) Polyethylene glycol octylphenyl ethers (Triton X-100), Polyethylene glycol alkyl ethers (Brij), Polypropylene glycol alkyl ethers, Glucoside alkyl ethers, and D-α-tocopherol polyethylene glycol succinate (vitamin E TPGS). The surfactant can be present at a concentration of about 0.0001% (w/v) to about 1% (w/v). In some pharmaceutical compositions as described, the surfactant is polyoxyethylenesorbitan monooleate (polysorbate 80) and is present at a concentration of about 0.01% (w/v).

In another example, disclosed herein is a method of formulating a therapeutic polypeptide, comprising

- a. a first concentration step, wherein the polypeptide in a first solution is concentrated using fed-batch ultrafiltration;
- b. a first solution exchange step, wherein the concentrated polypeptide in the first solution is exchanged into a second using diafiltration and three diavolumes of the second solution;
- c. a second concentration step, wherein the polypeptide in the second solution is concentrated using fed-batch ultrafiltration;
- d. a second solution exchange step, wherein the polypeptide in the concentrated second solution is exchanged into third solution using diafiltration and four diavolumes of the third solution;
- e. the temperature of the solution comprising the polypeptide is increased from about 25 °C to about 37 °C after the second solution exchange step; and
- f. a third concentration step, wherein the polypeptide is further concentrated using fed-batch ultrafiltration concentration;
- g. alternatively, a step adding polyoxyethylenesorbitan monooleate (polysorbate 80) at a concentration of about 0.01% (w/v) to the resulting solution of the third concentration step,

wherein the second and third solutions comprise a solution selected from the group consisting of a solution comprising about 140 mM N-acetyl arginine, about 50 mM Arg HCl, and about 10 mM sodium acetate, the solution having a pH of about 5.2; a solution comprising about 155 mM N-acetyl arginine, about 70 mM Arg HCl, and about 10 mM sodium acetate, the solution having a pH of about 5.4; and a solution comprising about 170 mM N-acetyl arginine, about 10 mM sodium acetate, the solution having a pH of about 5.6;

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

and

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;
- ii. a monoclonal antibody that competes with evolocumab for binding to PCSK9;
- iii. 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 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
 2. 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;

- iv. 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, 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, D238;
- 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 EGFA domain of LDLR.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a graph of the data from size-exclusion chromatography-high pressure liquid chromatography (SE-HPLC) of evolocumab samples at high concentrations in various formulations containing N-acetyl arginine following 1 month incubation at 40°C. Key to formulations indicated on x-axis: [acetate (mM)]/[N-acetyl arginine (mM)]/[proline (mM)]. Y-axis indicates percentage of sample that is high molecular weight (HMW) species (aggregates and oligomers of evolocumab).

Figure 2 shows a graph of a formulation viscosity comparison of evolocumab at 210 mg/mL – 1000 sec⁻¹ shear rate at 25 °C.

Figure 3A shows graphs produced by JMP Prediction Profiler software of SE-HPLC data for evolocumab formulation held at 40 °C for one month. **Figure 3B** shows similar data, except the samples were held at 25 °C for three months.

Figure 4 shows a bar graph of the percent HMW species by SE-HPLC of 210 mg/mL evolocumab formulations held at 40 °C for one month. Formulations vary by concentrations of N-acetyl arginine and arginine HCl concentration at varying pH. **Figures 5A-5C** show SE-HPLC chromatographs of selected pH 4.8 and pH 5.4 evolocumab samples held at the indicated temperatures and times (5 °C for six months (Fig. 5A); 25 °C for three months (Fig. 5B); and 40 °C for one month (Fig. 5C)) and compared to a

CLAIMS

1. A pharmaceutical composition comprising
 - 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;
 - ii. a monoclonal antibody that competes with evolocumab for binding to PCSK9;
 - iii. 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;
 - iv. 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, T187, H193, E195, I196, M201, V202, C223, T228, S235, G236, A239, G244, M247, I369, S372, C375, C378, R237, and D238;
 - 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;
 - b. N-acetyl arginine,
- wherein the pharmaceutical composition has a viscosity of at least less than about 80 cP.

2. The pharmaceutical composition of claim 1, wherein the PCSK9-binding polypeptide is a monoclonal antibody the comprises a heavy chain polypeptide comprising the following complementarity determining regions (CDRs):
 - a. heavy chain CDR1, CDR2, and CDR3 having an amino acid sequence of SEQ ID NOs:7, 8, and 9, respectively; and
 - b. light chain CDR1, CDR2, and CDR3 having an amino acid sequence of SEQ ID NOs:4, 5, and 6, respectively.
3. The pharmaceutical composition of claim 1, wherein the PCSK9-binding polypeptide is present at a concentration of about 140 mg/mL to about 260 mg/mL.
4. The pharmaceutical composition of claim 1 or 2, wherein the PCSK9-binding polypeptide concentration is about 210 mg/mL.
5. The pharmaceutical composition of any of claims 1-4, wherein the N-acetyl arginine is present at a concentration from about 25 mM to about 230 mM.
6. The pharmaceutical composition of any of claims 1-5, further comprising a buffer.
7. The pharmaceutical composition of any of claim 1-6, further comprising a surfactant.
8. The pharmaceutical composition of any of claims 1-7, further comprising proline.
9. The pharmaceutical composition of any of claims 1-8, further comprising an arginine salt.
10. The pharmaceutical composition of any of claims 1-9, wherein the composition comprises high molecular weight aggregates or oligomers of the PCSK9-binding polypeptide at less than about 3% of the PCSK9-binding polypeptide concentration.
11. The pharmaceutical composition of any of claims 1-11, wherein the pharmaceutical composition is liquid.
12. Use of the pharmaceutical composition of any of claims 1-11 in the manufacture of a medicament for treating a cholesterol related disorder in a subject in need thereof.
13. A kit, comprising the pharmaceutical composition of any of claims 1-11 and a delivery device selected from the group consisting of a syringe, an injector pen, a body injector, and an autoinjector.
14. 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;
- b. a monoclonal antibody that competes with evolocumab for binding to PCSK9;
- 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; 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;
- 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, T187, 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:
 - 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.

15. 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;
 - ii. a monoclonal antibody that competes with evolocumab for binding to PCSK9;
 - iii. 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;
 - iv. 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, D238, K243, S373, D374, S376, T377, F379, I154, T187, H193, E195, I196, M201, V202, C223, T228, S235, G236, A239, G244, M247, I369, S372, C375, C378, R237, D238;
 - 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.
16. The method of claim 14.e.ii, wherein the PCSK9-binding polypeptide that blocks binding of PCSK9 to LDLR is a monoclonal antibody that comprises a heavy chain polypeptide comprising the following complementarity determining regions (CDRs):
- a. heavy chain CDR1, CDR2, and CDR3 having an amino acid sequence of SEQ ID NOs: 7, 8, and 9, respectively; and

- b. light chain CDR1, CDR2, and CDR3 having an amino acid sequence of SEQ ID NOs: 4, 5, and 6, respectively.
- 17. The method of claim 14.e.ii, wherein before the third concentration step, the temperature of the solution comprising the polypeptide is increased from about 25 °C to about 37 °C.
- 18. The method of claim 14.e.ii, wherein in the first concentration step, the therapeutic polypeptide concentration is increased from about 3- to about 7-fold.
- 19. The method of claim 14.e.ii, wherein in the second concentration step, the therapeutic polypeptide concentration is increased from about 2- to 4-fold from the first concentration step.
- 20. The method of claim 14.e.ii, wherein in the third concentration step, the therapeutic polypeptide concentration is increased from about 1.5- to about 2-fold from the second concentration step.
- 21. The method of claim 14.e.ii, wherein the therapeutic polypeptide has a final concentration that is at least about 19-20-fold more concentrated than the initial concentration of the therapeutic polypeptide.
- 22. The method of claim 14.e.ii, wherein the concentration steps comprise fed-batch ultrafiltration.
- 23. The method of any of claims 15-22, wherein the second or third solution has a pH from about 4.8 to about 6.9.
- 24. 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 about 350 μm but less than or equal to about 500 μm ;
 - b. an open area that is greater than about 32% but less than or equal to about 36% of the membrane area;
 - c. a mesh count of less than about 16.2 n/cm but greater than or equal to about 12.2 n/cm;
 - d. a wire diameter that is greater than about 270 μm but less than or equal to about 340 μm ;
 - e. a basis weight that is greater than about 160 g/m^2 but less than or equal to 180 g/m^2 ;
 - f. a thickness greater than about 515 μm but less than or equal to about 610 μm ;
 - g. a membrane load of greater than about 1138.1 g/m^2 but less than or equal to about 1919.3 g/m^2 ; and

h. a maximum feed pressure of about 60 psi.

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