[0005] Defects in VWF are causal to Von Willebrand disease (VWD), which is characterized by a more or less pronounced bleeding phenotype. VWD type 3 is the most severe form in which VWF is completely missing, and VWD type 1 relates to a quantitative loss of VWF and its phenotype can be very mild. VWD type 2 relates to qualitative defects of VWF and can be as severe as VWD type 3. VWD type 2 has many sub forms, some being associated with the loss or the decrease of high molecular weight multimers. Von Willebrand syndrome type 2a (VWS-2A) is characterized by a loss of both intermediate and large multimers. VWS-2B is characterized by a loss of highest-molecular-weight multimers. Other diseases and disorders related to VWF are known in the art.

[0006] US. Patent Nos. 6,531,577, 7,166,709, and European Patent Application No. 04380188.5, describe plasma-derived VWF formulations. However, in addition to quantity and purity issues with plasma-derived VWF, there is also a risk of blood-born pathogens (e.g., viruses and Variant Creutzfeldt-Jakob disease (vCJD).

[0007] Thus there exists a need in the art to develop a stable pharmaceutical formulation comprising recombinant VWF.

SUMMARY

[0008] The present disclosure provides formulations useful for compositions comprising recombinant VWF, resulting in a highly stable pharmaceutical composition. The stable pharmaceutical composition is useful as a therapeutic agent in the treatment of individuals suffering from disorders or conditions that can benefit from the administration of recombinant VWF.

[0009] In one embodiment, the disclosure provides a stable liquid pharmaceutical formulation of a recombinant von Willebrand Factor (rVWF) comprising: (a) a rVWF; (b) a buffering agent; (c) one or more salts; (d) optionally a stabilizing agent; and (e) optionally a surfactant; wherein the rVWF comprises a polypeptide selected from the group consisting of: a) the amino acid sequence set out in SEQ ID NO: 3; b) a biologically active analog, fragment or variant of a); c) a polypeptide encoded by the polynucleotide set out in SEQ ID NO: 1; d) a biologically active analog, fragment or variant of c); and e) a polypeptide encoded by a polynucleotide that hybridizes to the polynucleotide set out in SEQ ID NO: 1 under moderately stringent hybridization conditions; wherein the buffer is comprised of a pH buffering agent in a range of about 0.1 mM to about 500 mM and wherein the pH is in a range of about 2.0 to about 12.0; wherein the salt is at a concentration of about 1 to 500 mM;

wherein the stabilizing agent is at a concentration of about 0.1 to 1000 mM; and wherein the surfactant is at a concentration of about 0.01 g/L to 0.5 g/L.

[0009a] In one aspect, there is provided a stable liquid pharmaceutical formulation of a recombinant von Willebrand Factor (rVWF) comprising: (a) a rVWF; (b) a buffering agent; (c) one or more salts; (d) a stabilizing agent; and (e) a surfactant;

wherein the rVWF is capable of causing agglutination of stabilized platelets in the presence of ristocetin;

wherein said rVWF comprises a polypeptide selected from the group consisting of:

a) the amino acid sequence set out in SEQ ID NO: 3;

b) a biologically active analog, fragment or variant of a);

c) a polypeptide encoded by the polynucleotide set out in SEQ ID NO: 1;

d) a biologically active analog, fragment or variant of c); and

e) a polypeptide encoded by a polynucleotide that hybridizes to the polynucleotide set out in SEQ ID NO: 1 under moderately stringent hybridization conditions;

wherein said buffer is comprised of a pH buffering agent in a range of 0.1 mM to 500 mM and wherein the pH is in a range of 2.0 to 12.0;

wherein said salt is at a concentration of 1 to 500 mM;

wherein said stabilizing agent is at a concentration of 0.1 to 1000 mM and is selected from the group consisting of mannitol, lactose, sorbitol, xylitol, sucrose, trehalose, mannose, maltose, lactose, glucose, raffinose, cellobiose, gentiobiose, isomaltose, arabinose, glucosamine, fructose, and combinations of these stabilizing agents; and

wherein said surfactant is at a concentration of 0.01 g/L to 0.5 g/L.

[0009b] In another aspect, there is provided a stable liquid pharmaceutical formulation of a recombinant von Willebrand Factor (rVWF) comprising: (a) a rVWF; (b) a buffering agent; (c) one or more salts; (d) a stabilizing agent; and (e) a optionally a surfactant;

wherein the rVWF is capable of causing agglutination of stabilized platelets in the presence of ristocetin;

wherein said rVWF comprises a polypeptide selected from the group consisting of:

a) the amino acid sequence set out in SEQ ID NO: 3;

b) a biologically active analog, fragment or variant of a);

c) a polypeptide encoded by the polynucleotide set out in SEQ ID NO: 1;

d) a biologically active analog, fragment or variant of c); and

e) a polypeptide encoded by a polynucleotide that hybridizes to the polynucleotide set out in SEQ ID NO: 1 under moderately stringent hybridization conditions;

wherein said buffer is comprised of a pH buffering agent in a range of 0.1 mM to 500 mM and wherein the pH is in a range of 2.0 to 12.0;

wherein said buffer comprises sodium citrate at a concentration of 15 mM;

wherein said salt is $CaCl_2$ at a concentration of 10 mM;
wherein said stabilizing agent is at a concentration of 0.1 to 1000 mM; and
wherein said surfactant is at a concentration of 0.01 g/L to 0.5 g/L.

[0010] In another embodiment, the aforementioned formulation is provided wherein the rVWF comprises the amino acid sequence set out in SEQ ID NO: 3. In another embodiment, an aforementioned formulation is provided wherein the buffering agent is selected from the group consisting of sodium citrate, glycine, histidine, Tris and combinations of these agents. In yet another embodiment, an aforementioned formulation is provided wherein the buffering agent is citrate. In still another embodiment of the disclosure, the aforementioned formulation is provided wherein pH is in the range of 6.0-8.0, or 6.5-7.3. In a related embodiment, the aforementioned formulation is provided wherein the pH is 7.0. In another embodiment, an aforementioned formulation is provided wherein the buffering agent is citrate and the pH is 7.0.

[0011] In still another embodiment, an aforementioned formulation is provided wherein the salt is selected from the group consisting of calcium chloride, sodium chloride and magnesium chloride. In another embodiment, the aforementioned formulation is provided wherein the salt is at a concentration range of 0.5 to 300 mM. In another embodiment, the aforementioned formulation is provided wherein the salt is calcium chloride at a concentration of 10 mM.

[0012] In another embodiment, an aforementioned formulation is provided wherein the rVWF comprises the amino acid sequence set out in SEQ ID NO: 3; wherein the buffering agent is citrate and the pH is 7.0; and wherein the salt is calcium chloride at a concentration of 10 mM. In still another embodiment, an aforementioned formulation is provided wherein the rVWF comprises the amino acid sequence set out in SEQ ID NO: 3; wherein the buffering agent is sodium citrate and the pH is 7.0; and wherein the salt is calcium chloride at a concentration of 10 mM and NaCl at a concentration of 100 mM.

[0013] Other formulations are also contemplated by the instant disclosure. For example, in one embodiment, an aforementioned formulation is provided wherein the one or more buffering agents is histidine and Tris at a concentration of 3.3 mM each. In another embodiment, the aforementioned formulation is provided wherein the pH is 7.0. In yet another embodiment, an aforementioned formulation is provided wherein the first salt is sodium chloride at a concentration of 30 mM and the second salt is calcium chloride at a concentration of 0.56 mM.

[0014] In still another embodiment of the disclosure, an aforementioned formulation is provided wherein the stabilizing agent is selected from the group consisting of mannitol, lactose, sorbitol, xylitol, sucrose, trehalose, mannose, maltose, lactose, glucose, raffinose, cellobiose,

gentiobiose, isomaltose, arabinose, glucosamine, fructose and combinations of these stabilizing agents. In another embodiment, the aforementioned formulation is provided wherein the stabilizing agents are trehalose at a concentration of 7.8 mM and mannitol at a concentration of 58.6 mM.

[0015] In another embodiment, an aforementioned formulation is provided wherein the surfactant is selected from the group consisting of digitonin, Triton X-100, Triton X-114, TWEEN-20, TWEEN-80 and combinations of these surfactants. In another embodiment, the aforementioned formulation is provided wherein the surfactant is TWEEN-80 at 0.03 g/L.

[0016] In one embodiment of the disclosure, an aforementioned formulation is provided wherein the rVWF comprises amino acid sequence set out in SEQ ID NO: 3; wherein the buffering agents are histidine at a concentration of 3.3 mM and Tris at a concentration of 3.3 mM at pH 7.0; wherein the first salt is sodium chloride at a concentration of 30 mM and the second salt is calcium chloride at a concentration of 0.56 mM; wherein the stabilizing agents are trehalose at a concentration of 7.8 mM mannitol at a concentration of 58.6 mM; and wherein the surfactant is TWEEN-80 at 0.03 g/L.

BRIEF DESCRIPTION OF THE FIGURES

[0017] Figure 1 shows that rVWF is not stable in Advate buffer after 26 weeks, due to the presence of glutathione.

[0018] Figure 2 shows that rVWF is stable in Advate 1:3 buffer for up to 12 weeks at 4 °C.

[0019] Figure 3 shows that the stability of a citrate-based formulation is better than Advate 1:3 buffer formulation containing 0.1M glutathione.

[0020] Figure 4 shows shows that rVWF concentration is stable over 26 weeks in Advate buffer.

[0021] Figure 5 shows that rVWF concentration is stable over time in Advate 1:3 buffer.

[0022] Figure 6 shows that rVWF concentration is stable over time in citrate-based buffer.

[0023] Figure 7 shows that most excipients increase the unfolding temperature of rVWF by about 1 or 2 °C.

[0024] Figure 8 shows that 10 mM $CaCl_2$ increases unfolding temperature of rVWF by about 8 °C to about 67 °C.

WHAT IS CLAIMED IS:

1. A stable liquid pharmaceutical formulation of a recombinant von Willebrand Factor (rVWF) comprising: (a) a rVWF; (b) a buffering agent; (c) one or more salts; (d) a stabilizing agent; and (e) a surfactant;

wherein the rVWF is capable of causing agglutination of stabilized platelets in the presence of ristocetin;

wherein said rVWF comprises a polypeptide selected from the group consisting of:

a) the amino acid sequence set out in SEQ ID NO: 3;

b) a biologically active analog, fragment or variant of a);

c) a polypeptide encoded by the polynucleotide set out in SEQ ID NO: 1;

d) a biologically active analog, fragment or variant of c); and

e) a polypeptide encoded by a polynucleotide that hybridizes to the polynucleotide set out in SEQ ID NO: 1 under moderately stringent hybridization conditions;

wherein said buffer is comprised of a pH buffering agent in a range of 0.1 mM to 500 mM and wherein the pH is in a range of 2.0 to 12.0;

wherein said salt is at a concentration of 1 to 500 mM;

wherein said stabilizing agent is at a concentration of 0.1 to 1000 mM and is selected from the group consisting of mannitol, lactose, sorbitol, xylitol, sucrose, trehalose, mannose, maltose, lactose, glucose, raffinose, cellobiose, gentiobiose, isomaltose, arabinose, glucosamine, fructose, and combinations of these stabilizing agents; and

wherein said surfactant is at a concentration of 0.01 g/L to 0.5 g/L.

2. The formulation of claim 1 wherein the buffering agent is selected from the group consisting of sodium citrate, glycine, histidine, Tris and combinations of these agents.

3. The formulation of claim 2 wherein the buffering agent is sodium citrate at a concentration of 15 mM.

4. The formulation of claim 3 wherein the pH is in the range of 6.0-8.0, and preferably the pH is in the range of 6.5-7.3.

5. The formulation of claim 4, wherein pH is 7.0.

6. The formulation of claim 1 wherein the buffering agent is citrate and the pH is 7.0.

7. The formulation of claim 1 wherein the salt is selected from the group consisting of calcium chloride, sodium chloride and magnesium chloride.

8. The formulation of claim 1 wherein the rVWF comprises the amino acid sequence set out in SEQ ID NO: 3; wherein the buffering agent is citrate and the pH is 7.0; and wherein the salt is calcium chloride at a concentration of 10 mM.

9. The formulation of claim 1 wherein the rVWF comprises the amino acid sequence set out in SEQ ID NO: 3; wherein the buffering agent is sodium citrate at a concentration of 15 mM and the pH is 7.0; and wherein the salt is calcium chloride at a concentration of 10 mM and sodium chloride of 100 mM.

10. The formulation of claim 2 wherein the one or more buffering agents is histidine and Tris at a concentration of 3.3 mM each.

11. The formulation of claim 7 wherein the one or more salt is sodium chloride at a concentration of 30 mM and calcium chloride at a concentration of 0.56 mM.

12. The formulation of claim 1 wherein the stabilizing agents are trehalose at a concentration of 7.8 mM and mannitol at a concentration of 58.6 mM.

13. The formulation of claim 1 wherein the surfactant is selected from the group consisting of digitonin, Triton X-100, Triton X-114, TWEEN-20, TWEEN-80 and combinations of these surfactants, and preferably the surfactant is TWEEN-80 at 0.03 g/L.

14. The formulation of claim 1 wherein the rVWF comprises the amino acid sequence set out in SEQ ID NO: 3; wherein the buffering agents are histidine at a concentration of 3.3 mM and Tris at a concentration of 3.3 mM at pH 7.0; wherein the salt is sodium chloride at a concentration of 30 mM and calcium chloride at a concentration of 0.56 mM; wherein the stabilizing agents are trehalose at a concentration of 7.8 mM and mannitol at a concentration of 58.6 mM; wherein the surfactant is TWEEN-80 at 0.03 g/L.

15. A stable liquid pharmaceutical formulation of a recombinant von Willebrand Factor (rVWF) comprising: (a) a rVWF; (b) a buffering agent; (c) one or more salts; (d) a stabilizing agent; and (e) a optionally a surfactant;

wherein the rVWF is capable of causing agglutination of stabilized platelets in the presence of ristocetin;

wherein said rVWF comprises a polypeptide selected from the group consisting of:

a) the amino acid sequence set out in SEQ ID NO: 3;

b) a biologically active analog, fragment or variant of a);

c) a polypeptide encoded by the polynucleotide set out in SEQ ID NO: 1;

d) a biologically active analog, fragment or variant of c); and

e) a polypeptide encoded by a polynucleotide that hybridizes to the polynucleotide set out in SEQ ID NO: 1 under moderately stringent hybridization conditions;

wherein said buffer is comprised of a pH buffering agent in a range of 0.1 mM to 500 mM and wherein the pH is in a range of 2.0 to 12.0;

wherein said buffer comprises sodium citrate at a concentration of 15 mM;

wherein said salt is $CaCl_2$ at a concentration of 10 mM;

wherein said stabilizing agent is at a concentration of 0.1 to 1000 mM; and

wherein said surfactant is at a concentration of 0.01 g/L to 0.5 g/L.

16. The formulation of claim 15 wherein said buffering agent further comprises glycine, histidine, Tris, or combinations of these agents.

17. The formulation of claim 15 or claim 16 wherein the pH is the range of 6.0-8.0, and preferably the pH is in the range of 6.5-7.3.

18. The formulation of claim 17 wherein the pH is 7.0.

19. The formulation of claim 15 wherein the stabilizing agent is selected from the group consisting of mannitol, lactose, sorbitol, xylitol, sucrose, trehalose, mannose, maltose, lactose, glucose, raffinose, cellobiose, gentiobiose, isomaltose, arabinose, glucosamine, fructose and combinations of these stabilizing agents.

20. The formulation of claim 15 wherein the surfactant is selected from the group consisting of digitonin, Triton X-100, Triton X-114, TWEEN-20, TWEEN-80 and combinations of these surfactants, and preferably the surfactant is TWEEN-80 at 0.03 g/L.

21. The formulation of claim 19 wherein the stabilizing agents are trehalose at a concentration of 7.8 mM and mannitol at a concentration of 58.6 mM.