

5 Factor VIII Formulation

BACKGROUND OF THE INVENTION

10 Factor VIII (FVIII) is a protein found in blood plasma, which acts as a cofactor in the cascade of reactions leading to blood coagulation. A deficiency in the amount of FVIII activity in the blood results in the clotting disorder known as hemophilia A, an inherited condition primarily affecting males. Hemophilia A is currently treated with therapeutic preparations of FVIII derived from human plasma or manufactured using recombinant DNA technology. Such
15 preparations are administered either in response to a bleeding episode (on-demand therapy) or at frequent, regular intervals to prevent uncontrolled bleeding (prophylaxis).

FVIII is known to be relatively unstable in therapeutic preparations. In blood plasma, FVIII is usually complexed with another plasma protein, von Willebrand factor (vWF), which is
20 present in plasma in a large molar excess of the VWF subunit to FVIII and is believed to protect FVIII from premature degradation. Another circulating plasma protein, albumin, may also play a role in stabilizing FVIII in vivo. Currently marketed FVIII preparations therefore often rely on the use of albumin and/or vWF to stabilize FVIII during the manufacturing process and during storage.

25 The albumin and vWF used in currently marketed FVIII preparations are derived from human blood plasma, however, and the use of such material has certain drawbacks. A large molar excess of albumin compared to FVIII is typically added in order to increase the stability of the FVIII in such preparations, and this makes it more difficult to characterize the FVIII protein
30 itself in these preparations. The addition of human-derived albumin to FVIII is also perceived as being a disadvantage with respect to recombinantly-produced FVIII preparations. This is because, in the absence of such added albumin, the theoretical risk of transmitting a virus would be reduced in recombinantly-derived FVIII preparations.

35 Several attempts to formulate FVIII without albumin or vWF (or with relatively low levels of these excipients) have been described. For example, U.S. Pat. No. 5,565,427 (EP 508 194) describes FVIII preparations which contain particular combinations of detergent and amino

acids, specifically arginine and glycine, in addition to excipients such as sodium chloride and sucrose.

U.S. Pat. No. 5,763,401 (EP 818 204) also describes a therapeutic FVIII formulation without
5 albumin, comprising 15-60 mM sucrose, up to 50 mM NaCl, up to 5 mM calcium chloride, 65-
400 mM glycine, and up to 50 mM histidine.

U.S. Pat. No. 5,733,873 (EP 627 924) to Osterberg (assigned to Pharmacia & Upjohn)
discloses formulations which include between 0.01-1 mg/ml of a surfactant.

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Other attempts to use low or high concentrations of sodium chloride have also been
described. U.S. Pat. No. 4,877,608 (EP 315 968) discloses formulations with relatively low
concentrations of sodium chloride, namely 0.5 mM to 15 mM NaCl.

15 On the other hand, U.S. Pat. No. 5,605,884 (EP 0 314 095) teaches the use of formulations
with relatively high concentrations of sodium chloride.

Further FVIII formulations are disclosed in WO 2010/054238, EP 1 712 223, WO
2000/48635, WO 96/30041, WO 96/22107, WO 2011/027152, EP 2 361 613, EP 0 410 207,
20 EP 0 511 234, US 5565427, EP 0 638 091, EP 0 871 476, EP 0 819 010, US 5874408, US
2005/0256038, US 2008/0064856, WO 2005/058283, WO 2012/037530 and WO
2014/026954. EP 1 712 223 A1 discloses FVIII compositions comprising histidine and
CaCl₂, but the combined concentrations of histidine and Ca²⁺ in the compositions of
EP 1 712 223 A1 are too low to confer the desired FVIII stability in aqueous solution.

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Other therapeutic FVIII formulations of the prior art generally include albumin and/or vWF for
the purpose of stabilizing FVIII and are therefore not relevant to the present disclosure.

One problem in the preparation of FVIII compositions is to provide a solution which is
30 suitable for lyophilization and use after subsequent reconstitution. An important criterion in
this regard is that the FVIII solution should show a stable FVIII activity and should show no
turbidity prior to lyophilization. If an aqueous FVIII solution is turbid prior to lyophilization this
is a clear sign that one or more substances in the solution, including FVIII itself, have
aggregated. This is highly undesirable, as it is typically an indication of denatured protein,
35 which leads to reduced activity. A second major issue with FVIII formulations is their stability
after lyophilization. Signs of poor stability include a reduced remaining activity of FVIII after

lyophilization, storage and reconstitution and the presence of high molecular weight complexes of FVIII.

5 Therefore, there is a great need for compositions comprising FVIII that do not show any turbidity or other relevant impairment of quality like e.g. significant loss of activity before lyophilization and possess long term stability after lyophilization at the same time. As the requirements of a stable solution prior to lyophilization and a stable lyophilisate in terms of composition may differ significantly, both conditions have to be considered when developing such a stable formulation.

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It has now been surprisingly discovered that this can be achieved by compositions according to this invention, which comprise histidine and calcium at certain minimum concentrations. The inventors particularly found that, with respect to FVIII stability, a lower concentration of histidine can be balanced by higher calcium concentrations, and *vice versa*. This is, for example, not disclosed in EP 1 712 223 A1, and, consequently, the compositions described therein do not anticipate the subject matter claimed in the present invention. Specifically, the histidine concentrations of the compositions of EP 1 712 223 A1 are lower than required by claim 1 of the present application. The compositions of the present invention have an additional advantage in that they are close to physiologic conditions in terms of osmolarity.

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20 This avoids irritations or other local effects that might be caused by significantly hyper-osmolaric compositions that are present in many prior art formulations of Factor VIII.

SUMMARY OF THE INVENTION

25 The present invention relates to an aqueous composition of FVIII, which composition may be used to treat a subject with hemophilia A.

The following items (1) to (93) describe various aspects and embodiments of the present invention:

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(1) An aqueous composition of coagulation Factor VIII, comprising:

- a. a FVIII molecule;
- b. 40 to 195 mM of a sodium salt;
- c. histidine;
- 35 d. at least 1 mM of a calcium salt; and
- e. a surfactant;

wherein $[\text{His}] \geq 180 \text{ mM} - 20 * [\text{Ca}^{2+}]$, wherein $[\text{Ca}^{2+}]$ is the concentration of calcium ions in the aqueous composition in millimole per liter, and $[\text{His}]$ is the concentration of histidine in the aqueous composition in millimole per liter; and wherein the osmolality of the composition is 600 mOsmol/L or less.

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(2) An aqueous composition of coagulation Factor VIII, comprising:

- a. a FVIII molecule;
- b. 40 to 95 mM of a sodium salt;
- c. histidine;
- 10 d. at least 1 mM of a calcium salt; and
- e. a surfactant;

wherein $[\text{His}] \geq 180 \text{ mM} - 20 * [\text{Ca}^{2+}]$, wherein $[\text{Ca}^{2+}]$ is the concentration of calcium ions in the aqueous composition in millimole per liter, and $[\text{His}]$ is the concentration of histidine in the aqueous composition in millimole per liter, and wherein the osmolality of the composition is
15 preferably 600 mOsmol/L or less.

(3) An aqueous composition of coagulation Factor VIII, comprising:

- a. a FVIII molecule;
- b. at least 40 mM of a sodium salt;
- 20 c. histidine;
- d. sucrose
- e. at least 1 mM of a calcium salt; and
- f. a surfactant;

wherein $[\text{His}] \geq 180 \text{ mM} - 20 * [\text{Ca}^{2+}]$, wherein $[\text{Ca}^{2+}]$ is the concentration of calcium ions in the
25 aqueous composition in millimole per liter, and $[\text{His}]$ is the concentration of histidine in the aqueous composition in millimole per liter, and wherein the osmolality of the composition is preferably 600 mOsmol/L or less.

(4) The aqueous composition according to any one of the above, wherein the formulae
30 are subject to the proviso that $[\text{His}] > 0$.

(5) The aqueous composition according to item (1) or (3), wherein the concentration of the sodium salt is 50 to 150 mM.

35 (6) The aqueous composition according to any one of the above, wherein the concentration of the sodium salt is 50 to 95 mM.

(7) The aqueous composition according to any one of the above, wherein the concentration of the sodium salt is 50 to 75 mM.

5 (8) The aqueous composition according to any one of the above, wherein the concentration of the sodium salt is 60 to 70 mM.

(9) The aqueous composition according to any one of the above, wherein the concentration of the sodium salt is about 65 mM.

10 (10) The aqueous composition according to any one of the above, wherein the concentration of histidine is at least 5 mM.

(11) The aqueous composition according to any one of the above, wherein the concentration of histidine is at least 10 mM.

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(12) The aqueous composition according to any one of the above, wherein the concentration of histidine is at least 20mM.

20 (13) The aqueous composition according to any one of the above, wherein the concentration of histidine is at least 30 mM.

(14) The aqueous composition according to any one of the above, wherein the concentration of histidine is at least 40 mM.

25 (15) The aqueous composition according to any one of the above, wherein the concentration of histidine is at least 50 mM.

(16) The aqueous composition according to any one of the above, wherein the concentration of histidine is 50 to 150 mM.

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(17) The aqueous composition according to any one of the above, wherein the concentration of histidine is about 100 mM.

(18) The aqueous composition according to any one of the above, wherein the composition has a pH from 5 to 9.

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(19) The aqueous composition according to any one of the above, wherein the composition has a pH from 6 to 8.

5 (20) The aqueous composition according to any one of the above, wherein the composition has a pH from 6.5 to 7.5.

(21) The aqueous composition according to any one of the above, wherein the composition has a pH from 6.8 to 7.2.

10 (22) The aqueous composition according to any one of the above, wherein the composition has a pH of about 7.

15 (23) The aqueous composition according to any one of the above, wherein the composition, upon (i) lyophilization, (ii) storage of the lyophilized composition at a temperature of +25°C and a relative humidity of 40% for a period of 6 months, and (iii) subsequent reconstitution of the lyophilized composition in distilled water, shows a recovery of FVIII:C activity of at least 80%, relative to the same composition upon (i) lyophilization and, without storage of the lyophilized composition, (ii) subsequent immediate reconstitution in distilled water.

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(24) The aqueous composition according to any one of the above, wherein the composition, upon (i) lyophilization, (ii) storage of the lyophilized composition at a temperature of +25°C and a relative humidity of 40% for a period of 12 months, and (iii) subsequent reconstitution of the lyophilized composition in distilled water, shows a recovery
25 of FVIII:C activity of at least 80%, relative to the same composition upon (i) lyophilization and, without storage of the lyophilized composition, (ii) subsequent immediate reconstitution in distilled water.

30 (25) The aqueous composition according to any one of the above, wherein the composition, upon (i) lyophilization, (ii) storage of the lyophilized composition at a temperature of +25°C and a relative humidity of 40% for a period of 18 months, and (iii) subsequent reconstitution of the lyophilized composition in distilled water, shows a recovery of FVIII:C activity of at least 80%, relative to the same composition upon (i) lyophilization and, without storage of the lyophilized composition, (ii) subsequent immediate reconstitution
35 in distilled water.

(26) The aqueous composition according to any one of the above, wherein the composition, upon (i) lyophilization, (ii) storage of the lyophilized composition at a temperature of +25°C and a relative humidity of 40% for a period of 24 months, and (iii) subsequent reconstitution of the lyophilized composition in distilled water, shows a recovery of FVIII:C activity of at least 80%, relative to the same composition upon (i) lyophilization and, without storage of the lyophilized composition, (ii) subsequent immediate reconstitution in distilled water.

(27) The aqueous composition according to any one of the above, wherein the composition, upon (i) lyophilization, (ii) storage of the lyophilized composition at a temperature of +25°C and a relative humidity of 40% for a period of 36 months, and (iii) subsequent reconstitution of the lyophilized composition in distilled water, shows a recovery of FVIII:C activity of at least 80%, relative to the same composition upon (i) lyophilization and, without storage of the lyophilized composition, (ii) subsequent immediate reconstitution in distilled water.

(28) The aqueous composition according to any one of the above, wherein the composition, upon (i) lyophilization, (ii) storage of the lyophilized composition at a temperature of +40°C and a relative humidity of 60% for a period of 6 months, and (iii) subsequent reconstitution of the lyophilized composition in distilled water, shows a recovery of FVIII:C activity of at least 80%, relative to the same composition upon (i) lyophilization and, without storage of the lyophilized composition, (ii) subsequent immediate reconstitution in distilled water.

(29) The aqueous composition according to any one of the above, wherein the composition, upon (i) lyophilization, (ii) storage of the lyophilized composition at a temperature of +40°C and a relative humidity of 60% for a period of 12 months, and (iii) subsequent reconstitution of the lyophilized composition in distilled water, shows a recovery of FVIII:C activity of at least 80%, relative to the same composition upon (i) lyophilization and, without storage of the lyophilized composition, (ii) subsequent immediate reconstitution in distilled water.

(30) The aqueous composition according to any one of the above, wherein the composition, upon (i) lyophilization, (ii) storage of the lyophilized composition at a temperature of +40°C and a relative humidity of 60% for a period of 18 months, and (iii) subsequent reconstitution of the lyophilized composition in distilled water, shows a recovery

of FVIII:C activity of at least 80%, relative to the same composition upon (i) lyophilization and, without storage of the lyophilized composition, (ii) subsequent immediate reconstitution in distilled water.

5 (31) The aqueous composition according to any one of the above, wherein the composition, upon (i) lyophilization, (ii) storage of the lyophilized composition at a temperature of +40°C and a relative humidity of 60% for a period of 24 months, and (iii) subsequent reconstitution of the lyophilized composition in distilled water, shows a recovery of FVIII:C activity of at least 80%, relative to the same composition upon (i) lyophilization
10 and, without storage of the lyophilized composition, (ii) subsequent immediate reconstitution in distilled water.

(32) The aqueous composition according to any one of the above, wherein the composition, upon (i) lyophilization, (ii) storage of the lyophilized composition at a
15 temperature of +25°C and a relative humidity of 40% for a period of 12 months, and (iii) subsequent reconstitution of the lyophilized composition in distilled water, has a turbidity of 18 NTU or less.

(33) The aqueous composition according to any one of the above, wherein the calcium salt is
20 calcium chloride.

(34) The aqueous composition according to any one of the above, wherein the sodium salt is sodium chloride.

25 (35) The aqueous composition according to any one of the above, wherein the composition further comprises a carbohydrate.

(36) The aqueous composition according to above (35), wherein the carbohydrate is selected from the group consisting of sucrose, trehalose and raffinose.
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(37) The aqueous composition according to above (35) or (36), wherein the carbohydrate is sucrose.

(38) The aqueous composition according to any one of above (35) to (37), wherein the concentration of the carbohydrate is 1 to 20 % w/w.
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(39) The aqueous composition according to any one of above (35) to (37), wherein the concentration of the carbohydrate is 2 to 10 % w/w.

5 (40) The aqueous composition according to any one of above (35) to (37), wherein the concentration of the carbohydrate is 3 to 8 % w/w.

(41) The aqueous composition according to any one of above (35) to (37), wherein the concentration of the carbohydrate is 4 to 6 % w/w.

10 (42) The aqueous composition according to any one of above (35) to (37), wherein the concentration of the carbohydrate is about 5 % w/w.

(43) The aqueous composition according to any one of the above, wherein the concentration of the surfactant is at least 0.001 % v/v.

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(44) The aqueous composition according to any one of the above, wherein the concentration of the surfactant is 0.001 to 0.1 % v/v.

20 (45) The aqueous composition according to any one of the above, wherein the concentration of the surfactant is 0.002 to 0.2 % v/v.

(46) The aqueous composition according to any one of the above, wherein the concentration of the surfactant is 0.005 % v/v.

25 (47) The aqueous composition according to any one of the above, wherein the surfactant is a non-naturally occurring surfactant.

(48) The aqueous composition according to any one of the above, wherein the surfactant is polysorbate 80.

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(49) The aqueous composition according to any one of the above, wherein the surfactant is polysorbate 20.

(50) The aqueous composition according to any one of the above, wherein the composition further comprises at least one amino acid other than histidine.

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(51) The aqueous composition according to above (50), wherein the at least one amino acid other than histidine is selected from the group consisting of arginine, asparagine, aspartic acid, glutamic acid, glutamine, lysine, methionine, phenylalanine, leucine, isoleucine and combinations thereof.

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(52) The aqueous composition according to above (50), wherein the at least one amino acid other than histidine is arginine.

(53) The aqueous composition according to above (50), wherein the at least one amino acid other than histidine is asparagine.

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(54) The aqueous composition according to above (50), wherein the at least one amino acid other than histidine is aspartic acid.

(55) The aqueous composition according to above (50), wherein the at least one amino acid other than histidine is glutamic acid.

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(56) The aqueous composition according to above (50), wherein the at least one amino acid other than histidine is glutamine.

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(57) The aqueous composition according to above (50), wherein the at least one amino acid other than histidine is lysine.

(58) The aqueous composition according to above (50), wherein the at least one amino acid other than histidine is methionine.

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(59) The aqueous composition according to above (50), wherein the at least one amino acid other than histidine is phenylalanine.

(60) The aqueous composition according to above (50), wherein the at least one amino acid other than histidine is isoleucine.

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(61) The aqueous composition according to any one of the above (50) to (60), wherein the concentration of the at least one amino acid other than histidine is at least 0.1 mM.

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(62) The aqueous composition according to any one of the above (50) to (60), wherein the concentration of the at least one amino acid other than histidine is at least 1 mM.

(63) The aqueous composition according to any one of the above (50) to (60), wherein the
5 concentration of the at least one amino acid other than histidine is 5 to 300 mM.

(64) The aqueous composition according to any one of the above (50) to (60), wherein the concentration of the at least one amino acid other than histidine is 10 to 200 mM.

10 (65) The aqueous composition according to any one of the above, wherein the composition further comprises at least one antioxidant.

(66) The aqueous composition according to above (65), wherein the at least one antioxidant is selected from the group consisting of reduced glutathione, methionine,
15 cysteine, sodium sulfite, vitamin A, vitamin E, ascorbic acid, sodium ascorbate and combinations thereof.

(67) The aqueous composition according to above (65), wherein the at least one antioxidant is reduced glutathione.

20 (68) The aqueous composition according to above (65), wherein the at least one antioxidant is methionine.

(69) The aqueous composition according to above (65), wherein the at least one antioxidant is cysteine.

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(70) The aqueous composition according to above (65), wherein the at least one antioxidant is sodium sulfite.

(71) The aqueous composition according to above (65), wherein the at least one
30 antioxidant is vitamin A.

(72) The aqueous composition according to above (65), wherein the at least one antioxidant is vitamin E.

(73) The aqueous composition according to above (65), wherein the at least one
35 antioxidant is ascorbic acid.

(74) The aqueous composition according to above (65), wherein the at least one antioxidant is sodium ascorbate.

(75) The aqueous composition according to any one of the above (65) to (74), wherein the
5 concentration of the at least one antioxidant is at least 0.05 mM.

(76) The aqueous composition according to any one of the above (65) to (74), wherein the concentration of the at least one antioxidant is 0.05 to 100 mM.

10 (77) The aqueous composition according to any one of the above (65) to (74), wherein the concentration of the at least one antioxidant is 0.1 to 20 mM.

(78) The aqueous composition according to any one of the above (65) to (74), wherein the concentration of the at least one antioxidant is 0.25 to 5 mM.

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(79) The aqueous composition according to any one of the above, wherein the Factor VIII molecule is a non-naturally occurring FVIII molecule.

(80) The aqueous composition according to (79), wherein the Factor VIII molecule has
20 been recombinantly produced.

(81) The aqueous composition according to (79) or (80), wherein the Factor VIII molecule has a glycosylation pattern different from that of human plasma-derived FVIII.

25 (82) The aqueous composition according to an one of the above (79) to (81), wherein the FVIII molecule has an amino acid sequence different from SEQ ID NO:1.

(83) The aqueous composition according to an one of the above (79) to (82), wherein the FVIII molecule is selected from the group consisting of (i) B-domain deleted or truncated
30 FVIII molecules, (ii) single-chain FVIII molecules, (iii) FVIII molecules having protective groups or half-life extending moieties, (iv) fusion proteins comprising a FVIII amino acid sequence fused to a heterologous amino acid sequence, and (v) combinations thereof.

(84) The aqueous composition according to any one of the above, wherein the Factor VIII molecule has the amino acid sequence as shown in SEQ ID NO:2.

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(85) The aqueous composition according to any one of the above, wherein the composition has a turbidity of 18 NTU or less, preferably of 6 NTU or less, more preferably of 3 NTU or less.

5 (86) The aqueous composition according to the above (85), wherein the composition, prior to lyophilization, has a turbidity of 18 NTU or less, preferably of 6 NTU or less, more preferably of 3 NTU or less.

10 (87) The aqueous composition according to the above (85), wherein the composition, upon lyophilization and reconstitution in distilled water, has a turbidity of 18 NTU or less, preferably of 6 NTU or less, more preferably of 3 NTU or less.

(88) A composition obtainable by lyophilizing the aqueous composition according to any one of the above.

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(89) An aqueous composition obtainable by reconstituting the lyophilized aqueous composition according to above (88) with an aqueous solution.

20 (90) The aqueous composition of (89), wherein the aqueous solution is selected from distilled water, a salt solution and a solution comprising histidine.

(91) A method of stabilizing a FVIII molecule, comprising mixing the components defined in any one of the above (1) to (3) to obtain an aqueous composition, and lyophilizing the aqueous composition.

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(92) The method of (91), wherein the aqueous composition obtained is a composition according to any one of the above (1) to (87).

30 (93) A method of treating a blood coagulation disorder, comprising administering to a subject a pharmaceutically effective amount of the composition of the above (89) or (90).

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 depicts the relationship between the concentrations of histidine and calcium ions in the composition of the present invention. The figure summarizes the results of a liquid formulation study (Example 7) with varying histidine and calcium chloride concentrations whereas the other excipients were kept constant (65 mmol/L sodium chloride, 5% sucrose, 0.005% Tween[®] 80). Dots represent a turbidity above a threshold level of 18 NTU, diamonds represent a clear solution (turbidity less than or equal to 18 NTU). The graph illustrates that if certain concentrations of histidine and calcium ions are applied, a clear solution is resulting. The requirement for a clear solution is represented by a formula given later.

DETAILED DESCRIPTION OF INVENTION

Factor VIII molecules

As used herein, the term "Factor VIII" or "FVIII" refers to molecules having at least part of the coagulation activity of human native Factor VIII. Human FVIII consists of 2351 amino acids (including a signal peptide) and 2332 amino acids (without the signal peptide). "Human native FVIII" is the human plasma-derived FVIII molecule having the full length sequence as shown in SEQ ID NO: 1 (amino acid 1-2332). The detailed domain structure, A1-a1-A2-a2-B-a3-A3-C1-C2 has the corresponding amino acid residues (referring to SEQ ID NO 1) : A1 (1-336), a1 (337-372), A2 (373-710), a2 (711-740), B (741-1648), a3 (1649-1689), A3 (1690-2020), C1 (2021-2173) and C2 (2174-2332).

The coagulation activity of the FVIII molecule can be determined using a one-stage clotting assay (e.g. as described in Lee et al., Thrombosis Research 30, 511 519 (1983)) or a chromogenic substrate assay (e.g. the coamatic FVIII test kit from Chromogenix-Instrumentation Laboratory SpA V. le Monza 338 - 20128 Milano, Italy). Further details of these activity assays are described *infra*.

Preferably, the FVIII molecules used in accordance with this invention have at least 10% of the specific molar activity of human native FVIII. The term "specific molar activity" refers to the coagulation activity per mole of FVIII and is indicated in "IU/mole FVIII".

In a preferred embodiment, the FVIII molecule is a non-naturally occurring FVIII molecule. Preferably, the non-naturally occurring FVIII molecule has been produced recombinantly. In another embodiment, the FVIII molecule has been produced in cell culture. In another preferred embodiment, the non-naturally occurring FVIII molecule has a glycosylation pattern different from that of plasma-derived FVIII. In yet another embodiment, the FVIII molecule is

selected from the group consisting of (i) B-domain deleted or truncated FVIII molecules, (ii) single-chain FVIII molecules, (iii) recombinantly produced two-chain FVIII molecules, (iv) FVIII molecules having protective groups or half-life extending moieties, (v) fusion proteins comprising a FVIII amino acid sequence fused to a heterologous amino acid sequence, and
5 (vi) combinations thereof.

The terms "Factor VIII" and "FVIII" are used synonymously herein. "Factor VIII compositions" in the sense of the present invention include compositions comprising FVIII and FVIIIa. FVIIIa may typically be present in small amounts, e.g. about 1 to 2% FVIIIa, referred to the
10 total amount of FVIII protein in the composition. "FVIII" includes natural allelic variations of FVIII that may exist and occur from one individual to another. FVIII may be plasma-derived or recombinantly produced, using well known methods of production and purification. The degree and location of glycosylation, tyrosine sulfation and other post-translation modifications may vary, depending on the chosen host cell and its growth conditions.

15 The term FVIII includes FVIII analogues. The term "FVIII analogue" as used herein refers to a FVIII molecule (full-length or B-domain- truncated/deleted) wherein one or more amino acids have been substituted or deleted compared to SEQ ID NO 1 or, for B-domain truncated/deleted FVII molecules, the corresponding part of SEQ ID NO 1. FVIII analogues
20 do not occur in nature but are obtained by human manipulation.

The Factor VIII molecules included in the compositions of the present invention may also be B-domain-truncated/deleted FVIII molecules wherein the remaining domains correspond to the sequences as set forth in amino acid numbers 1-740 and 1649-2332 of SEQ ID NO: 1.
25 Other forms of B-domain deleted FVIII molecules have additionally a partial deletion in their a3 domain, which leads to single-chain FVIII molecules.

It follows that these FVIII molecules are recombinant molecules produced in transformed host cells, preferably of mammalian origin. However, the remaining domains in a B-domain
30 deleted FVIII, (i.e. the three A- domains, the two C-domains and the a1, a2 and a3 regions) may differ slightly e.g. about 1%, 2%, 3%, 4% or 5% from the respective amino acid sequence as set forth in SEQ ID NO 1 (amino acids 1-740 and 1649-2332).

The FVIII molecules included in the composition of the present invention may be two-chain
35 FVIII molecules or single-chain FVIII molecules. The FVIII molecules included in the composition of the present invention may also be biologically active fragments of FVIII, i.e.,

FVIII wherein domain(s) other than the B-domain has/have been deleted or truncated, but wherein the FVIII molecule in the deleted/truncated form retains its ability to support the formation of a blood clot. FVIII activity can be assessed in vitro using techniques well known in the art. A preferred test for determining FVIII activity according to this invention is the chromogenic substrate assay or the one stage assay (*see infra*). Amino acid modifications (substitutions, deletions, etc.) may be introduced in the remaining domains, e.g., in order to modify the binding capacity of Factor VIII with various other components such as e.g. Von Willebrand Factor (vWF), low density lipoprotein receptor- related protein (LPR), various receptors, other coagulation factors, cell surfaces, etc. or in order to introduce and/or abolish glycosylation sites, etc. Other mutations that do not abolish FVIII activity may also be accommodated in a FVIII molecule/analogue for use in a composition of the present invention.

FVIII analogues also include FVIII molecules, in which one or more of the amino acid residues of the parent polypeptide have been deleted or substituted with other amino acid residues, and/or wherein additional amino acid residues has been added to the parent FVIII polypeptide.

Furthermore, the Factor VIII molecules/analogues may comprise other modifications in e.g. the truncated B-domain and/or in one or more of the other domains of the molecules ("FVIII derivatives"). These other modifications may be in the form of various molecules conjugated to the Factor VIII molecule, such as e.g. polymeric compounds, peptidic compounds, fatty acid derived compounds, etc.

The term FVIII includes glycopegylated FVIII. In the present context, the term "glycopegylated FVIII" is intended to designate a Factor VIII molecule (including full length FVIII and B-domain truncated/deleted FVIII) wherein one or more PEG group(s) has/have been attached to the FVIII polypeptide via the polysaccharide sidechain(s) (glycan(s)) of the polypeptide.

The term FVIII includes FVIII molecules having protective groups or half-life extending moieties. The terms "protective groups"/"half-life extending moieties" is herein understood to refer to one or more chemical groups attached to one or more amino acid site chain functionalities such as -SH, -OH, -COOH, -CONH₂, -NH₂, or one or more N- and/or O-glycan structures and that can increase in vivo circulatory half-life of a number of therapeutic proteins/peptides when conjugated to these proteins/peptides. Examples of protective

groups/half-life extending moieties include: Biocompatible fatty acids and derivatives thereof, Hydroxy Alkyl Starch (HAS) e.g. Hydroxy Ethyl Starch (HES), Poly (Gly_x-Ser_y)_n (Homo Amino acid Polymer (HAP)), Hyaluronic acid (HA), Heparosan polymers (HEP), Phosphorylcholine-based polymers (PC polymer), Fleximer[®] polymers (Mersana Therapeutics, MA, USA),
5 Dextran, Poly-sialic acids (PSA), polyethylene glycol (PEG), an Fc domain, Transferrin, Albumin, Elastin like peptides, XTEN[®] polymers (Amunix, CA, USA), Albumin binding peptides, a von Willebrand factor fragment (vWF fragment), a Carboxyl Terminal Peptide (CTP peptide, Prolor Biotech, IL), and any combination thereof (see, for example, McCormick, C.L., A.B. Lowe, and N. Ayres, Water-Soluble Polymers, in Encyclopedia of
10 Polymer Science and Technology. 2002, John Wiley & Sons, Inc.). The manner of derivatization is not critical and can be elucidated from the above.

The FVIII molecules which can be used in accordance with this invention include fusion proteins comprising a FVIII amino acid sequence fused to a heterologous amino acid
15 sequence, preferably a half-life extending amino acid sequence. Preferred fusion proteins are Fc fusion proteins and albumin fusion proteins. The term "Fc fusion protein" is herein meant to encompass FVIII fused to an Fc domain that can be derived from any antibody isotype. An IgG Fc domain will often be preferred due to the relatively long circulatory half-life of IgG antibodies. The Fc domain may furthermore be modified in order to modulate certain
20 effector functions such as e.g. complement binding and/or binding to certain Fc receptors. Fusion of FVIII with an Fc domain, which has the capacity to bind to FcRn receptors, will generally result in a prolonged circulatory half-life of the fusion protein compared to the half-life of the wt FVIII. It follows that a FVIII molecule for use in the present invention may also be a derivative of a FVIII analogue, such as, for example, a fusion protein of an FVIII
25 analogue, a PEGylated or glycoPEGylated FVIII analogue, or a FVIII analogue conjugated to a heparosan polymer. The term "albumin fusion protein" is herein meant to encompass FVIII fused to an albumin amino acid sequence or a fragment or derivative thereof. The heterologous amino acid sequence may be fused to the N- or C-terminus of FVIII, or it may be inserted internally within the FVIII amino acid sequence. The heterologous amino acid
30 sequence may be any "half life extending polypeptide" described in WO 2008/077616 A1, the disclosure of which is incorporated herein by reference.

Examples of FVIII molecules for use in compositions of the present invention comprise for instance the FVIII molecules described in WO 2010/045568, WO 2009/062100,
35 WO 2010/014708, WO 2008/082669, WO 2007/126808, US 2010/0173831, US 2010/0173830, US 2010/0168391, US 2010/0113365, US 2010/0113364,

WO 2003/031464, WO 2009/108806, WO 2010/102886, WO 2010/115866,
WO 2011/101242, WO 2011/101284, WO 2011/101277, WO 2011/131510,
WO 2012/007324, WO 2011/101267, WO 2013/083858, and WO 2004/067566.

5 Examples of FVIII molecules, which can be used in a composition of the present invention include the active ingredient of Advate®, Helixate®, Kogenate®, Xyntha® as well as the FVIII molecule described in WO 2008/135501, WO 2009/007451 and the construct designated "dBN(64-53)" of WO 2004/067566. This construct has the amino acid sequence shown in SEQ ID NO 2.

10

The concentration of Factor VIII in the composition of the present invention is typically in the range of 10-10,000 IU/mL. In different embodiments, the concentration of FVIII molecules in the compositions of the invention is in the range of 10-8,000 IU/mL, or 10-5,000 IU/mL, or 20-3,000 IU/mL, or 50-1,500 IU/mL, or 3,000 IU/mL, or 2,500 IU/mL, or 2,000 IU/mL, or 1,500
15 IU/mL, or 1,200 IU/mL, 1,000 IU/mL, or 800 IU/mL, or 600 IU/mL, or 500 IU/mL, or 400 IU/mL, or 300 IU/mL, or 250 IU/mL, or 200 IU/mL, or 150 IU/mL, or 100 IU/mL.

"International Unit," or "IU," is a unit of measurement of the blood coagulation activity (potency) of FVIII as measured by a FVIII activity assay such as a one stage clotting assay
20 or a chromogenic substrate FVIII activity assay using a standard calibrated against an international standard preparation calibrated in "IU". One stage clotting assays are known to the art, such as that described in N Lee, Martin L, et al., An Effect of Predilution on Potency Assays of FVIII Concentrates, Thrombosis Research (Pergamon Press Ltd.) 30, 511 519 (1983). Principle of the one stage assay: The test is executed as a modified version of the
25 activated Partial Thromboplastin Time (aPTT)-assay: Incubation of plasma with phospholipids and a surface activator leads to the activation of factors of the intrinsic coagulation system. Addition of calcium ions triggers the coagulation cascade. The time to formation of a measurable fibrin clot is determined. The assay is executed in the presence of Factor VIII deficient plasma. The coagulation capability of the deficient plasma is restored by
30 Coagulation Factor VIII included in the sample to be tested. The shortening of coagulation time is proportional to the amount of Factor VIII present in the sample. The activity of Coagulation Factor VIII is quantified by direct comparison to a standard preparation with a known activity of Factor VIII in International Units.

35 Another standard assay is a chromogenic substrate assay. Chromogenic substrate assays may be purchased commercially, such as the coamatic FVIII test kit (Chromogenix-

Instrumentation Laboratory SpA V. le Monza 338 - 20128 Milano, Italy). Principle of the chromogenic assay: In the presence of calcium and phospholipid, Factor X is activated by Factor IXa to Factor Xa. This reaction is stimulated by Factor VIIIa as cofactor. FVIIIa is formed by low amounts of thrombin in the reaction mixture from FVIII in the sample to be measured. When using the optimum concentrations of Ca^{2+} , phospholipid and Factor IXa and an excess quantity of Factor X, activation of Factor X is proportional to the potency of Factor VIII. Activated Factor X releases the chromophore pNA from the chromogenic substrate S-2765. The release of pNA, measured at 405 nm, is therefore proportional to the amount of FXa formed, and, therefore, also to the Factor VIII activity of the sample.

Freeze-drying or lyophilization, unless otherwise indicated by the context in which it appears, shall be used to denote a drying process in which a solution of materials (i.e. an active pharmaceutical ingredient and various formulation additives or "excipients") is converted into a solid. A typical freeze-drying process consists of three stages, "freezing", "primary drying" and "secondary drying". In the freezing stage almost all contained water is converted into ice and solutes into solids (crystalline or amorphous). In the primary drying stage the ice is removed from the product by direct sublimation which is achieved by maintaining a favorable pressure gradient between the water molecules (ice) and the surrounding atmosphere. In the secondary drying stage residual moisture is removed from the product by desorption.

If concentrations (w/v) are given for freeze-dried compositions they refer to the volume directly prior to freeze-drying.

Unless otherwise noted, percentage terms express weight/weight percentages and temperatures are in the Celsius scale.

Osmolarity

The Factor VIII composition of the present invention typically has an osmolarity of less than 0.6 Osm/L. This is closer to physiological conditions than the osmolarities in many prior art formulations that contain high concentrations of sodium chloride. In different embodiments of the invention, the formulation has an osmolarity of below 0.55 Osm/L, below 0.5 Osm/L, below 0.45 Osm/L, or below 0.4 Osm/L. The terms "osmolarity" and "osmotic concentration" are used interchangeably herein.

Osmolarity, or osmotic concentration, is the measure of solute concentration, defined as the number of osmoles (Osm) of solute per litre (L) of solution (osmol/L or Osm/L). Whereas

molarity measures the number of moles of solute per unit volume of solution, osmolarity measures the number of osmoles of solute particles per unit volume of solution.

5 The theoretical calculation of osmolarity is well known to the person skilled in the art. Briefly, one calculates for each component of the solution the product of the osmotic coefficient f , the number of particles n into which the molecule dissociates in water, and the molar concentration, and sums the result over all components. The osmolarity of a solution can be calculated from the following expression: $\text{Osm/L} = \sum_i f_i n_i C_i$, where the index i represents the identity of a particular component; f_i is the osmotic coefficient for a particular component; n is
10 the number of particles into which the molecule dissociates in water; C is the molar concentration of the component. The molar concentration has some temperature dependence; for the present purpose it is referred to the concentrations at 25 °C.

15 An alternative way to assess the osmotic pressure that a solution may exert after injection is by evaluating the osmolality, in which the content of the components is evaluated relative to solvent mass. Osmolality and osmolarity can easily be interconverted if the density of the solution and the dry mass of the dissolved components are known. Osmolality can be measured by a number of methods, most commonly freezing point depression.

20 For example, for water, 1 Osmol of a solute added to 1 kg of water lowers the freezing point by 1.86°C. Methods for measuring the osmolality of a solution by freezing point depression are described, for example, in the European Pharmacopeia 2.2.35 and the U.S. Pharmacopeia chapter 785.

25 The table below lists the osmotic coefficients and number of particles n for some important excipients. For other components, a value of $f=1$ provides a sufficiently good approximation for practical purposes, and the value of n is well known for essentially all compounds relevant for pharmaceutical preparations for parenteral use.

Excipient	f	n
NaCl	0.93	2
CaCl ₂	0.86	3
Sucrose	1.02	1
Histidine	1.0	1
L-Methionine	1.0	1
Poloxamer-188	1.0	1
Polysorbate 20	1.0	1
Polysorbate 80	1.0	1

Salts

The composition according to the present invention comprises a sodium salt.

5

In one embodiment the composition of the present invention contains at least 40 mM of a sodium salt (e.g. NaCl). In a series of embodiments, the composition comprises 40 to 195 mM of a sodium salt (e.g. NaCl), or 45 to 180 mM, or 45 to 150 mM, or 50 to 125 mM or 50 to 100 mM, or 60 to 95 mM, or 60 to 75 mM, e.g. about 65 mM. In yet another embodiment the concentration of sodium salt (e.g. NaCl) is 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94 or 95 mM.

10

Preferably, the sodium salt is sodium chloride; in another embodiment, the salt is sodium acetate. In a third embodiment, the composition of the invention contains a mixture of sodium chloride and sodium acetate.

15

The composition according to the present invention further comprises a calcium salt. The calcium salt used in accordance with the present invention is typically soluble in water so that it dissociates into one or more calcium ions and counter-anions upon dissolution in an aqueous solution. The term "Ca²⁺" is synonymous to the term "calcium ion".

20

In one embodiment the calcium concentration is defined by the following formula, wherein $[Ca^{2+}]$ is the concentration of calcium ions in millimole per liter, and $[His]$ is the concentration of histidine in millimole per liter:

$$[Ca^{2+}] \geq 9 \text{ mM} - 0.05*[His]$$

5

In another embodiment the calcium concentration is defined by the following formula, wherein $[Ca^{2+}]$ is the concentration of calcium ions in millimole per liter, and $[His]$ is the concentration of histidine in millimole per liter:

$$[Ca^{2+}] \geq 9.5 \text{ mM} - 0.05*[His]$$

10

In another embodiment the calcium concentration is defined by the following formula, wherein $[Ca^{2+}]$ is the concentration of calcium ions in millimole per liter, and $[His]$ is the concentration of histidine in millimole per liter:

$$[Ca^{2+}] \geq 10 \text{ mM} - 0.05*[His]$$

15

In another embodiment the calcium concentration is defined by the following formula, wherein $[Ca^{2+}]$ is the concentration of calcium ions in millimole per liter, and $[His]$ is the concentration of histidine in millimole per liter:

$$[Ca^{2+}] \geq 10.5 \text{ mM} - 0.05*[His]$$

20

The above formulae are subject to the proviso that $[Ca^{2+}] > 0$. Typically, the composition contains at least 1 mM, preferably at least 2 mM, more preferably at least 4 mM, most preferably at least 5 mM of a calcium salt. In another embodiment the composition contains up to 100 mM of a calcium salt. In another embodiment, the composition comprises 1-50 mM of a calcium salt, or 2-40 mM, or 3-30 mM, or 4-20 mM. In one particular embodiment, the composition contains about 10 mM of a calcium salt.

25

The calcium salt may, for example, be selected from the group of calcium chloride, calcium acetate, calcium gluconate, calcium lactate, calcium benzoate and mixtures thereof, and other soluble calcium salts well known by the person skilled in the art. In a preferred embodiment, the calcium salt is calcium chloride.

30

Histidine

The composition according to the present invention comprises histidine, preferably L-histidine.

- 5 In one embodiment the histidine concentration is defined by the following formula, wherein $[Ca^{2+}]$ is the concentration of calcium ions in millimole per liter, and [His] is the concentration of histidine in millimole per liter:

$$[His] \geq 180 \text{ mM} - 20*[Ca^{2+}]$$

- 10 In another embodiment the histidine concentration is defined by the following formula, wherein $[Ca^{2+}]$ is the concentration of calcium ions in millimole per liter, and [His] is the concentration of histidine in millimole per liter:

$$[His] \geq 190 \text{ mM} - 20*[Ca^{2+}]$$

- 15 In another embodiment the histidine concentration is defined by the following formula, wherein $[Ca^{2+}]$ is the concentration of calcium ions in millimole per liter, and [His] is the concentration of histidine in millimole per liter:

$$[His] \geq 200 \text{ mM} - 20*[Ca^{2+}]$$

- 20 In another embodiment the histidine concentration is defined by the following formula, wherein $[Ca^{2+}]$ is the concentration of calcium ions in millimole per liter, and [His] is the concentration of histidine in millimole per liter:

$$[His] \geq 210 \text{ mM} - 20*[Ca^{2+}]$$

- 25 The above formulae are subject to the proviso that $[His] > 0$. Typically, the concentration of histidine is at least 5 mM, preferably at least 10 mM, preferably at least 20 mM, preferably 20-400 mM, preferably at least 25 mM, preferably 25-200 mM, preferably at least 50 mM, preferably 50-300 mM, preferably 55-170 mM, preferably 60-150 mM, preferably 65-120 mM, more preferably 50-100 mM, e.g. about 100 mM. In yet another embodiment the
30 concentration of histidine is 10, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300 or 400 mM.

Surfactant

The composition according to the present invention comprises a surfactant. As used herein, "surfactants" refers to agents which protect the active substance from air-to-solution interface or solution-to-surface interface induced stress or damage. Preferably, the surfactant is a non-naturally occurring surfactant.

Typical non-naturally occurring surfactants (with examples of trade names given in brackets []) are polyoxyethylene sorbitan fatty acid esters such as polyoxyethylene (20) sorbitan monolaurate [Tween[®] 20], polyoxyethylene (20) sorbitan monopalmitate [Tween[®] 40] or polyoxyethylene (20) sorbitan monooleate [Tween[®] 80], poloxamers such as polyoxypropylene-polyoxyethylene block copolymer [Pluronic[®] F68 / poloxamer 188], polyethylene glycol octylphenyl ether [Triton[®] X-100] or polyoxyethyleneglycol dodecyl ether [Brij[®] 35] . The use of a surfactant in aqueous compositions is well-known to the skilled person. For convenience reference is made to Remington: The Science and Practice of Pharmacy, 19th edition, 1995. In one embodiment of the invention, the composition comprises sorbitan monooleate [Tween[®] 80]. In certain embodiments, the composition of the present invention comprises a surfactant which is not sensitive to oxidation, e.g. alkyl saccharides. In another embodiment, the surfactant is a vegetable-derived surfactant, and/or it is free from petrochemicals, and/or it has been produced without using petrochemicals.

In one embodiment, the concentration of the surfactant is at least 0.001 % v/v, preferably 0.001 - 0.1 % v/v, preferably 0.002 - 0.3 % v/v, more preferably 0.003 to 0.1 % v/v, and most preferably about 0.005 % v/v. In yet another embodiment the concentration of the surfactant is 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.5, 0.7, 0.8, 0.9 or 1.0 % v/v. In one embodiment, the concentration of Polyoxyethylene (20) sorbitan monooleate [Tween[®] 80] is at least 0.001 % v/v, preferably 0.001 - 0.1 % v/v, preferably 0.002 - 0.3 % v/v, more preferably 0.003 to 0.1% v/v, and most preferably about 0.005 % v/v.

Buffer

The composition according to the invention may comprise a buffering agent. As used herein, "buffering agents" or "buffers" refers to agents which maintain the pH of the preparation in a range where administration of the preparation (e.g. intravenous or subcutaneous) is normally well tolerated (e.g. pH 5 to 9) and, furthermore, avoid pH shifts and protect the active substance from pH induced stress or damage during lyophilization. Typical examples for

substances used as buffer substances are e.g. carbonate, phosphate, citrate, borate, 2-amino-2-hydroxymethyl-1,3-propanediol [=TRIS], histidine, glycine.

5 The buffer (or buffering agents) may be selected from the group consisting of acetate, benzoate, carbonate, citrate, glycylglycine, Hepes, histidine, glycine, and TRIS, bicine, tricine, succinate, aspartic acid, glutamic acid or mixtures thereof. In one embodiment of the invention, the concentration of the buffering substance is 1 to 200 mM, such as, e.g., 1 to 150 mM or 1 to 50 mM or 1 to 25 mM or 5 to 20 mM or 5 to 15 mM.

10 The composition of the invention typically has a pH from 4 to 10. In another embodiment, the composition has a pH of 5.0 to 9.0 or 6.0 to 8.0 or 6.5 to 7.5 or 6.8 to 7.2 or about 7. In other embodiments, the pH of the composition is 5.5 ± 0.2 , 5.6 ± 0.2 , 5.7 ± 0.2 , 5.8 ± 0.2 , 5.9 ± 0.2 , 6.0 ± 0.2 , 6.1 ± 0.2 , 6.2 ± 0.2 , 6.3 ± 0.2 , 6.4 ± 0.2 , 6.5 ± 0.2 , 6.6 ± 0.2 , 6.7 ± 0.2 , 6.8 ± 0.2 , 6.9 ± 0.2 , 7.0 ± 0.2 , 7.1 ± 0.2 , 7.2 ± 0.2 , 7.3 ± 0.2 , 7.4 ± 0.2 , or 7.5 ± 0.2 . In other embodiments, the pH of the
15 composition is 5.5 ± 0.1 , 5.6 ± 0.1 , 5.7 ± 0.1 , 5.8 ± 0.1 , 5.9 ± 0.1 , 6.0 ± 0.1 , 6.1 ± 0.1 , 6.2 ± 0.1 , 6.3 ± 0.1 , 6.4 ± 0.1 , 6.5 ± 0.1 , 6.6 ± 0.1 , 6.7 ± 0.1 , 6.8 ± 0.1 , 6.9 ± 0.1 , 7.0 ± 0.1 , 7.1 ± 0.1 , 7.2 ± 0.1 , 7.3 ± 0.1 , 7.4 ± 0.1 , or 7.5 ± 0.1 . In yet other embodiments, the pH of the stabilizing formulation is 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0.

20

Carbohydrates

The composition of the invention may further comprise a carbohydrate. As used herein, "carbohydrates" refers to sugars, such as mono-, di-, or polysaccharides, or water-soluble glucans, including for example fructose, glucose, mannose, mannitol, sorbose, sorbit, xylose,
25 maltose, lactose, sucrose, galactose, dextran, trehalose, pullulan, dextrin, cyclodextrin, soluble starch, hydroxyethyl starch, and carboxymethylcellulose may be used.

The carbohydrate may, for example, be selected from the group of mono-, di-, or polysaccharides (including for example the monosaccharides fructose, glucose, mannose,
30 galactose, the disaccharides lactose, sucrose, trehalose, maltose and the polysaccharides dextran, raffinose, stachyose).

In one embodiment of the invention, the FVIII composition comprises one or more carbohydrates from the group of: sucrose, raffinose, trehalose, or mixtures thereof. In one
35 embodiment, the FVIII composition comprises a carbohydrate in a concentration of 2 to 10 % w/w, preferably 3 to 8 % w/w, preferably 4 to 6 % w/w and most preferably 5 % w/w. In yet

another embodiment the concentration of the carbohydrate is 2, 3, 4, 5, 6, 7, 8, 9 or 10 % w/w.

5 In specific embodiments the carbohydrate is sucrose. In one embodiment, the FVIII composition comprises sucrose in a concentration of 2 - 10 % w/w, preferably 3 - 8 % w/w, preferably 4 - 6 % w/w and most preferably 5 % w/w. In yet another embodiment the concentration of sucrose is 2, 3, 4, 5, 6, 7, 8, 9 or 10 % w/w.

10 In specific embodiments the carbohydrate is trehalose. In one embodiment, the FVIII composition comprises trehalose in a concentration of 2 to 10 % w/w, preferably 3 to 8 % w/w, preferably 4 to 6 % w/w and most preferably 5 % w/w. In yet another embodiment the concentration of trehalose is 2, 3, 4, 5, 6, 7, 8, 9 or 10 % w/w.

15 In specific embodiments the carbohydrate is raffinose. In one embodiment, the FVIII composition comprises raffinose in a concentration of 2 to 10 % w/w, preferably 3 to 8 % w/w, preferably 4 to 6 % w/w and most preferably 5 % w/w. In yet another embodiment the concentration of raffinose is 2, 3, 4, 5, 6, 7, 8, 9 or 10 % w/w.

Amino acids

20 In one embodiment the composition of the invention further comprises one or more amino acids other than histidine. As used herein, "amino acids" refers to any natural or non-natural pharmaceutically acceptable amino acid. Non-limiting examples of amino acids include, isoleucine, alanine, leucine, asparagine, lysine, aspartic acid, methionine, cysteine, phenylalanine, glutamic acid, threonine, glutamine, tryptophan, glycine, valine, proline,
25 selenocysteine, serine, tyrosine, arginine, histidine, ornithine, taurine, and the like. The amino acid other than histidine is preferably selected from the group consisting of arginine, asparagine, aspartic acid, glutamic acid, glutamine, lysine, methionine, phenylalanine, isoleucine, leucine and combinations thereof.

30 In one embodiment the aqueous composition comprises arginine and isoleucine.

In one embodiment the aqueous composition comprises arginine, glutamic acid and phenylalanine.

35 In one embodiment the aqueous composition comprises arginine, glutamic acid, phenylalanine and methionine.

In one embodiment the aqueous composition comprises arginine, isoleucine and methionine.

In one embodiment the aqueous composition comprises glutamine, glutamic acid, isoleucine and methionine.

5

In one embodiment the concentration of the amino acid other than histidine is at least 0.2 mM, preferably 1 to 300 mM, preferably 5 to 300 mM, preferably 10 to 200 mM, preferably 55 to 150 mM, preferably 25 to 100 mM, preferably 40 to 180 mM, preferably 50 to 90 mM. In yet another embodiment the concentration of the amino acid other than histidine is 0.2, 0.5,
10 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195 or 200 mM.

In one embodiment the concentration of the arginine is at least 1 mM, preferably 5 to 300 mM, preferably 10 to 200 mM, preferably 55 to 150 mM, preferably 25 to 100 mM, preferably 40 to 180 mM, preferably 50 to 90 mM. In yet another embodiment the concentration of the arginine is 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195 or 200 mM.

20

In one embodiment the concentration of the isoleucine is at least 1 mM, preferably 5 to 300 mM, preferably 10 to 200 mM, preferably 55 to 150 mM, preferably 25 to 100 mM, preferably 40 to 180 mM, preferably 50 to 90 mM. In yet another embodiment the concentration of the isoleucine is 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95,
25 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195 or 200 mM.

In one embodiment the concentration of the glutamic acid is at least 1 mM, preferably 5 to 300 mM, preferably 10 to 200 mM, preferably 55 to 150 mM, preferably 25 to 100 mM, preferably 40 to 180 mM, preferably 50 to 90 mM. In yet another embodiment the concentration of the glutamic acid is 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195 or 200 mM.

35 In one embodiment the concentration of the phenylalanine is at least 1 mM, preferably 5 to 200 mM, preferably 10 to 160 mM, preferably 55 to 150 mM, preferably 25 to 100 mM,

preferably 40 to 180 mM, preferably 50 to 90 mM. In yet another embodiment the concentration of the phenylalanine is 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195 or 200 mM.

5

Antioxidants

The composition of the present invention may further contain one or more antioxidants(s). An "antioxidant" within the meaning of the present invention is a pharmaceutically compatible compound or composition that decelerates, inhibits, interrupts and/or stops oxidation processes. Antioxidants include, in particular, the following substances: tocopherols and the esters thereof, reduced glutathione, methionine, monothioglycerol, cysteine, homocysteine, cystathionine, vitamin A, sesamol of sesame oil, coniferyl benzoate of benzoin resin, nordihydroguaietic resin and nordihydroguaiaretic acid (NDGA), gallates (among others, methyl, ethyl, propyl, amyl, butyl, lauryl gallates), butylated hydroxyanisole (BHAIBHT, also butyl-p-cresol); ascorbic acid and salts and esters thereof (for example ascorbyl palmitate), erythorbic acid (isoascorbic acid) and salts and esters thereof, monothioglycerol, sodium formaldehyde sulfoxylate, sodium metabisulfite, sodium bisulfite, sodium sulfite, potassium metabisulfite, butylated hydroxyanisole, butylated hydroxytoluene (BHT), propionic acid. Typical antioxidants are tocopherol such as, for example, α -tocopherol and the esters thereof, butylated hydroxytoluene and butylated hydroxyanisole. The terms "tocopherol" also includes esters of tocopherol. A known tocopherol is α -tocopherol. The term " α -tocopherol" includes esters of α -tocopherol (for example, α -tocopherol acetate). In one embodiment of the invention the antioxidant is methionine, e.g., L- methionine.

25 In one embodiment, the concentration of the antioxidant is at least 0.05 mM, preferably 0.05 to 100 mM, preferably 0.1 to 80 mM, preferably 0.2 to 50 mM, preferably 0.5 to 70 mM, preferably 1 to 25 mM, preferably 0.1 to 20 mM, preferably 0.25 to 1 mM. In yet another embodiment the concentration of the antioxidant is 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 13.0, 14.0, 15.0, 16.0, 17.0, 18.0, 19.0, 20.0, 25.0, 30.0, 35.0, 40.0, 45.0, 50.0, 55.0, 60.0, 65.0, 70.0, 75.0, 80.0, 85.0, 90.0, 95.0 or 100.0 mM.

In one embodiment, the concentration of methionine is at least 0.05 mM, preferably 0.05 to 100 mM, preferably 0.1 to 80 mM, preferably 0.2 to 50 mM, preferably 0.5 to 70 mM, preferably 1 to 25 mM, preferably 0.1 to 20 mM, preferably 0.25 to 5 mM. In yet another embodiment the concentration of methionine is 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9,

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1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 13.0, 14.0, 15.0, 16.0, 17.0, 18.0, 19.0, 20.0, 25.0, 30.0, 35.0, 40.0, 45.0, 50.0, 55.0, 60.0, 65.0, 70.0, 75.0, 80.0, 85.0, 90.0, 95.0 or 100.0 mM.

5 In one embodiment, the concentration of glutathione is at least 0.05 mM, preferably 0.05 to 100 mM, preferably 0.1 to 80 mM, preferably 0.2 to 50 mM, preferably 0.5 to 70 mM, preferably 1 to 25 mM, preferably 0.1 to 20 mM, preferably 0.25 to 1 mM. In yet another embodiment the concentration of glutathione is 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 13.0, 14.0, 15.0, 16.0, 17.0, 18.0, 19.0, 20.0, 25.0, 30.0, 35.0, 40.0, 45.0, 50.0, 55.0, 60.0, 65.0, 70.0, 75.0, 80.0, 85.0, 90.0, 95.0 or 100.0 mM.

Stabilizing agents

The composition of the present invention may comprise a stabilizing agent. As used herein, a "stabilizing agent" refers to a chemical (other synonyms are e.g. "co-solvent", "co-solute", "chemical additive; "excipient") which aids in the stabilization of a labile therapeutic agent in an aqueous formulation either in solution state or during freeze-thawing as well as freeze-drying and subsequent storage of the dehydrated lyophilisate. Examples of suitable stabilizing agents for the formulations and methods provided herein include, without limitation, buffering agents (e.g., TRIS, HEPES, amino acids, etc.), osmolytes (e.g., sugars, sugar alcohols, etc.), cryoprotectants/lyoprotectants (e.g alcohols such as glycerol, methanol, isopropanol; sugars such as sucrose, xylitol, dextrose, trehalose; carboxylic acids and carboxylates such as lactic acid and lactates, malic acid and maleates; PEGs such as ethylene glycol, PEG 200, PEG 2 000, PEG 20 000; Polymers such as polyvinylpyrrolidons, PVP 12, PVP 17, PVP 30; bulking agents (e.g., amino acids, polyols such as mannitol etc.), salts, polymers, surfactants, antioxidants and the like.

The concentration of the stabilizing agent depends on the chemical nature of the agent and can be chosen in accordance with the disclosure hereinabove.

Stability

In a specific embodiment of the compositions provided above, the composition is stable. The term "stable" as used herein means that the potency of the composition, as measured by a FVIII activity assay, after storage for a period of time, is at least 80% of the potency prior to storage. Unless indicated otherwise herein, the stability testing is carried out as described in the document CPMP/ICH/2736/99 from the European Medicines Agency titled "NOTE FOR

GUIDANCE ON STABILITY TESTING: STABILITY TESTING OF NEW DRUG SUBSTANCES AND PRODUCTS” as of August 2003.

In a series of embodiments, the composition provided herein upon lyophilization will be stable for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48 or more months (25°C; 40% relative humidity [RH]). In a preferred embodiment, the lyophilized composition will be stable for at least 6 months (25°C; 40% RH). In a more preferred embodiment, the lyophilized composition will be stable for at least 12 months (25°C; 40% RH). In another preferred embodiment, the lyophilized composition will be stable for at least 18 months (25°C; 40% RH). In another preferred embodiment, the lyophilized composition will be stable for at least 24 months (25°C; 40% RH). In another preferred embodiment, the lyophilized composition will be stable for at least 36 months (25°C; 40% RH).

In a specific embodiment of the compositions provided above, the lyophilized composition is stable for at least 6 months, preferably for at least 12 months, more preferably for at least 24 months, even more preferably for at least 36 months when stored at 25°C / 40% RH.

In another embodiment of the compositions provided above, the lyophilized composition is stable for at least 6 months, preferably for at least 12 months, more preferably for at least 24 months, even more preferably for at least 36 months when stored at 30°C / 40% RH.

In another embodiment of the compositions provided above, the lyophilized composition is stable for at least 6 months, preferably for at least 12 months, more preferably for at least 24 months when stored at 40°C / 40% RH.

In another embodiment of the compositions provided above, the lyophilized composition is stable for at least 6 months, preferably for at least 12 months, more preferably for at least 24 months, even more preferably for at least 36 months when stored at 25°C / 60% RH.

In another embodiment of the compositions provided above, the lyophilized composition is stable for at least 6 months, preferably for at least 12 months, more preferably for at least 24 months, even more preferably for at least 36 months when stored at 30°C / 60% RH.

In another embodiment of the compositions provided above, the lyophilized composition is stable for at least 6 months, preferably for at least 12 months, more preferably for at least 24 months when stored at 40°C / 60% RH.

- 5 In another embodiment of the compositions provided above, the lyophilized composition is stable for at least 6 months, preferably for at least 12 months, more preferably for at least 24 months, even more preferably for at least 36 months when stored at 25°C / 65% RH.

- 10 In another embodiment of the compositions provided above, the lyophilized composition is stable for at least 6 months, preferably for at least 12 months, more preferably for at least 24 months, even more preferably for at least 36 months when stored at 30°C / 65% RH.

- 15 In another embodiment of the compositions provided above, the lyophilized composition is stable for at least 6 months, preferably for at least 12 months, more preferably for at least 24 months when stored at 40°C / 65% RH.

- 20 In another embodiment of the compositions provided above, the lyophilized composition is stable for at least 6 months, preferably for at least 12 months, more preferably for at least 24 months, even more preferably for at least 36 months when stored at 25°C / 75% RH.

- In another embodiment of the compositions provided above, the lyophilized composition is stable for at least 6 months, preferably for at least 12 months, more preferably for at least 24 months, even more preferably for at least 36 months when stored at 30°C / 75% RH.

- 25 In another embodiment of the compositions provided above, the lyophilized composition is stable for at least 6 months, preferably for at least 12 months, more preferably for at least 24 months when stored at 40°C / 75% RH.

- 30 In another embodiment of the compositions provided above, the lyophilized composition is stable for at least 6 months, preferably for at least 12 months, more preferably for at least 24 months, even more preferably for at least 36 months when stored at 25°C / 25% RH.

- 35 In another embodiment of the compositions provided above, the lyophilized composition is stable for at least 6 months, preferably for at least 12 months, more preferably for at least 24 months, even more preferably for at least 36 months when stored at 30°C / 25% RH.

In another embodiment of the compositions provided above, the lyophilized composition is stable for at least 6 months, preferably for at least 12 months, more preferably for at least 24 months when stored at 40°C / 25% RH.

- 5 Preferably, the amount of high molecular weight components, as determined by SE-HPLC, after storage of the lyophilized composition under one of the conditions defined *supra* is 3% or less. The amount of high molecular weight components, as determined by SE-HPLC as described in the examples of this application.
- 10 In one embodiment the composition is stable for at least 48 hours, preferably for at least 72 hours, more preferably for at least 96 hours, most preferably for at least one week, when stored in the liquid state at 4°C. The aqueous composition typically retains at least 80% of its Factor VIII activity for at least 48 hours, preferably for at least 72 hours, more preferably for at least 96 hours, most preferably for at least one week, when stored in the liquid state at 2 to
- 15 8°C. This applies to the aqueous composition prior to lyophilization and/or to the aqueous composition after lyophilization and reconstitution in an aqueous solution, e.g. in distilled water.

In a specific embodiment of the compositions provided above, the composition retains at

20 least 85% of its Factor VIII activity for at least 48 hours, preferably for at least 72 hours, more preferably for at least 96 hours, most preferably for at least one week, when stored in the liquid state at 4°C. This applies to the aqueous composition prior to lyophilization and/or to the aqueous composition after lyophilization and reconstitution in an aqueous solution, e.g. in distilled water.

25 In a specific embodiment of the compositions provided above, the composition retains at least 90% of its Factor VIII activity for at least 48 hours, preferably for at least 72 hours, more preferably for at least 96 hours, most preferably for at least one week, when stored in the liquid state at 4°C. This applies to the aqueous composition prior to lyophilization and/or to

30 the aqueous composition after lyophilization and reconstitution in an aqueous solution, e.g. in distilled water.

In a specific embodiment of the invention, the composition shows little or no turbidity, e.g. prior to lyophilization or upon lyophilization and reconstitution in water. As used herein, the

35 term "turbidity" refers to the opalescence of a solution. Turbidity can be determined visually or instrumentally, as described in Pharm. Eur. 8.0, section 2.2.1."Clarity and degree of

opalescence of liquids". Any reference to turbidity in the present application refers to the methods described in Pharm. Eur. 8.0, section 2.2.1."Clarity and degree of opalescence of liquids".

5 In one embodiment, the composition of the present invention has a turbidity of 18 NTU or less. In another embodiment, the composition of the present invention has a turbidity of 6 NTU or less. In yet another embodiment, the composition of the present invention has a turbidity of 3 NTU or less. These turbidity values are fulfilled prior to lyophilization and after lyophilization and reconstitution in distilled water.

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In yet another embodiment, the composition of the present invention is clear. In the present application, a composition or solution is deemed to be "clear" when its opalescence is not more pronounced than that of "reference suspension III" described in Pharm. Eur. 8.0, section 2.2.1."Clarity and degree of opalescence of liquids". When referring herein to a composition having "no turbidity" or being "free of turbidity", it is to be understood that the composition is "clear" in accordance with this definition.

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Preferred are the following compositions (table 1) comprising:

	FVIII [IU/ml]	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Tween® 80 [% w/w]	Sucrose [% w/w]
composition 1	100	52	100	10	0.004	5
composition 2	100	40	100	10	0.005	5
composition 3	100	50	100	10	0.005	5
composition 4	100	60	100	10	0.005	5
composition 5	100	70	100	10	0.005	5
composition 6	100	80	100	10	0.005	5
composition 7	100	95	100	10	0.005	5
composition 8	100	50	100	7	0.005	5
composition 9	100	50	100	10	0.005	5
composition 10	100	50	100	15	0.005	5
composition 11	100	50	100	20	0.005	5
composition 12	100	65	100	10	0.005	5
composition 13	100	65	100	5.5	0.005	5
composition 14	100	65	55	10	0.005	5
composition 15	100	65	30	10	0.005	5
composition 16	100	65	10	10	0.005	5

Further preferred are the following compositions (table 2) comprising:

	FVIII [IU/ml]	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Tween® 80 [% w/w]	Sucrose [% w/w]
composition 17	200	52	100	10	0.004	5
composition 18	200	40	100	10	0.005	5
composition 19	200	50	100	10	0.005	5
Composition 20	200	60	100	10	0.005	5
composition 21	200	70	100	10	0.005	5
composition 22	200	80	100	10	0.005	5
composition 23	200	95	100	10	0.005	5
composition 24	200	50	100	7	0.005	5
composition 25	200	50	100	10	0.005	5
composition 27	200	50	100	15	0.005	5
composition 28	200	50	100	20	0.005	5
composition 29	200	65	100	10	0.005	5
composition 30	200	65	100	5.5	0.005	5
composition 31	200	65	55	10	0.005	5
composition 32	200	65	30	10	0.005	5
composition 33	200	65	10	10	0.005	5

Further preferred are the following compositions (table 3) comprising:

	FVIII [IU/ml]	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Tween®80 [% w/w]	Sucrose [% w/w]
composition 34	400	52	100	10	0.004	5
composition 35	400	40	100	10	0.005	5
composition 36	400	50	100	10	0.005	5
composition 37	400	60	100	10	0.005	5
composition 38	400	70	100	10	0.005	5
composition 39	400	80	100	10	0.005	5
composition 40	400	95	100	10	0.005	5
composition 41	400	50	100	7	0.005	5
composition 42	400	50	100	10	0.005	5
composition 43	400	50	100	15	0.005	5
composition 44	400	50	100	20	0.005	5
composition 45	400	65	100	10	0.005	5
composition 46	400	65	100	5.5	0.005	5
composition 47	400	65	55	10	0.005	5
composition 48	400	65	30	10	0.005	5
composition 49	400	65	10	10	0.005	5

Further preferred are the following compositions (table 4) comprising:

	FVIII [IU/ml]	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Tween® 80 [% w/w]	Sucrose [% w/w]
composition 50	800	52	100	10	0.004	5
composition 51	800	40	100	10	0.005	5
composition 52	800	50	100	10	0.005	5
composition 53	800	60	100	10	0.005	5
composition 54	800	70	100	10	0.005	5
composition 55	800	80	100	10	0.005	5
composition 56	800	95	100	10	0.005	5
composition 57	800	50	100	7	0.005	5
composition 58	800	50	100	10	0.005	5
composition 59	800	50	100	15	0.005	5
composition 60	800	50	100	20	0.005	5
composition 61	800	65	100	10	0.005	5
composition 62	800	65	100	5.5	0.005	5
composition 63	800	65	55	10	0.005	5
composition 64	800	65	30	10	0.005	5
composition 65	800	65	10	10	0.005	5

Further preferred are the following compositions (table 5) comprising:

	FVIII [IU/ml]	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Tween® 80 [% w/w]	Sucrose [% w/w]
composition 66	1200	52	100	10	0.004	5
composition 67	1200	40	100	10	0.005	5
composition 68	1200	50	100	10	0.005	5
composition 69	1200	60	100	10	0.005	5
composition 70	1200	70	100	10	0.005	5
composition 71	1200	80	100	10	0.005	5
composition 72	1200	95	100	10	0.005	5
composition 73	1200	50	100	7	0.005	5
composition 74	1200	50	100	10	0.005	5
composition 75	1200	50	100	15	0.005	5
composition 76	1200	50	100	20	0.005	5
composition 77	1200	65	100	10	0.005	5
composition 78	1200	65	100	5.5	0.005	5
composition 79	1200	65	55	10	0.005	5
composition 80	1200	65	30	10	0.005	5
composition 81	1200	65	10	10	0.005	5

Preferred are the following compositions (table 6) comprising:

	FVIII [IU/ml]	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Tween® 80 [% w/w]	Sucrose [% w/w]
composition 82	1500	52	100	10	0.004	5
composition 83	1500	40	100	10	0.005	5
composition 84	1500	50	100	10	0.005	5
composition 85	1500	60	100	10	0.005	5
composition 86	1500	70	100	10	0.005	5
composition 87	1500	80	100	10	0.005	5
composition 88	1500	95	100	10	0.005	5
composition 89	1500	50	100	7	0.005	5
composition 90	1500	50	100	10	0.005	5
composition 91	1500	50	100	15	0.005	5
composition 92	1500	50	100	20	0.005	5
composition 93	1500	65	100	10	0.005	5
composition 94	1500	65	100	5.5	0.005	5
composition 95	1500	65	55	10	0.005	5
composition 96	1500	65	30	10	0.005	5
composition 97	1500	65	10	10	0.005	5

Preferred are the following compositions (table 7) comprising:

	FVIII [IU/ml]	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Tween®80 [% w/w]	Sucrose [% w/w]
composition 98	2500	52	100	10	0.004	5
composition 99	2500	40	100	10	0.005	5
composition 100	2500	50	100	10	0.005	5
composition 101	2500	60	100	10	0.005	5
composition 102	2500	70	100	10	0.005	5
composition 103	2500	80	100	10	0.005	5
composition 104	2500	95	100	10	0.005	5
composition 105	2500	50	100	7	0.005	5
composition 106	2500	50	100	10	0.005	5
composition 107	2500	50	100	15	0.005	5
composition 108	2500	50	100	20	0.005	5
composition 109	2500	65	100	10	0.005	5
composition 110	2500	65	100	5.5	0.005	5
composition 111	2500	65	55	10	0.005	5
composition 112	2500	65	30	10	0.005	5
composition 113	2500	65	10	10	0.005	5

Preferred are the following compositions (table 8) comprising:

	FVIII [IU/ml]	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Tween® 80 [% w/w]	Sucrose [% w/w]	Arginine [mM]	Isoleucine [mM]	Glutamate [mM]	Phenylalanin [mM]	Methionine [mM]	Glutathione [mM]
composition 114	100	80	100	10	0.005	5	25	25	--	--	--	--
Composition 115	100	80	100	10	0.005	5	25	25	--	--	50	--
composition 116	100	80	100	10	0.005	5	25	--	25	25	--	--
composition 117	100	80	100	10	0.005	5	25	--	25	25	50	--
composition 118	100	80	100	10	0.005	5	25	25	--	--	--	5
composition 119	100	80	100	10	0.005	5	25	--	25	25	50	5
composition 120	100	80	100	10	0.005	5	100	--	100	50	50	--
composition 121	100	80	100	10	0.005	5	100	--	100	50	50	5

Preferred are the following compositions (table 9) comprising:

	FVIII [IU/ml]	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Tween®80 [% w/w]	Sucrose [% w/w]	Arginine [mM]	Isoleucine [mM]	Glutamate [mM]	Phenylalanine [mM]	Methionine [mM]	Glutathione [mM]
composition 122	200	80	100	10	0.005	5	25	25	--	--	--	--
composition 123	200	80	100	10	0.005	5	25	25	--	--	50	--
composition 124	200	80	100	10	0.005	5	25	--	25	25	--	--
composition 125	200	80	100	10	0.005	5	25	--	25	25	50	--
composition 126	200	80	100	10	0.005	5	25	25	--	--	--	5
composition 127	200	80	100	10	0.005	5	25	--	25	25	50	5
composition 128	200	80	100	10	0.005	5	100	--	100	50	50	--
composition 129	200	80	100	10	0.005	5	100	--	100	50	50	5

Preferred are the following compositions (table 10) comprising:

	FVIII [IU/ml]	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Tween®80 [% w/w]	Sucrose [% w/w]	Arginine [mM]	Isoleucine [mM]	Glutamate [mM]	Phenylalanine [mM]	Methionine [mM]	Glutathione [mM]
composition 130	400	80	100	10	0.005	5	25	25	--	--	--	--
composition 131	400	80	100	10	0.005	5	25	25	--	--	50	--
composition 132	400	80	100	10	0.005	5	25	--	25	25	--	--
composition 133	400	80	100	10	0.005	5	25	--	25	25	50	--
composition 134	400	80	100	10	0.005	5	25	25	--	--	--	5
composition 135	400	80	100	10	0.005	5	25	--	25	25	50	5
composition 136	400	80	100	10	0.005	5	100	--	100	50	50	--
composition 137	400	80	100	10	0.005	5	100	--	100	50	50	5

Preferred are the following compositions (table 11) comprising:

	FVIII [IU/ml]	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Tween®80 [% w/w]	Sucrose [% w/w]	Arginine [mM]	Isoleucine [mM]	Glutamate [mM]	Phenylalanin [mM]	Methionine [mM]	Glutathione [mM]
composition 138	800	80	100	10	0.005	5	25	25	--	--	--	--
composition 139	800	80	100	10	0.005	5	25	25	--	--	50	--
composition 140	800	80	100	10	0.005	5	25	--	25	25	--	--
composition 141	800	80	100	10	0.005	5	25	--	25	25	50	--
composition 142	800	80	100	10	0.005	5	25	25	--	--	--	5
composition 143	800	80	100	10	0.005	5	25	--	25	25	50	5
composition 144	800	80	100	10	0.005	5	100	--	100	50	50	--
composition 145	800	80	100	10	0.005	5	100	--	100	50	50	5

Preferred are the following compositions (table 12) comprising:

	FVIII [IU/ml]	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Tween®80 [% w/w]	Sucrose [% w/w]	Arginine [mM]	Isoleucine [mM]	Glutamate [mM]	Phenylalanin [mM]	Methionine [mM]	Glutathione [mM]
composition 146	1200	80	100	10	0.005	5	25	25	--	--	--	--
composition 147	1200	80	100	10	0.005	5	25	25	--	--	50	--
composition 148	1200	80	100	10	0.005	5	25	--	25	25	--	--
composition 149	1200	80	100	10	0.005	5	25	--	25	25	50	--
composition 150	1200	80	100	10	0.005	5	25	25	--	--	--	5
composition 151	1200	80	100	10	0.005	5	25	--	25	25	50	5
composition 152	1200	80	100	10	0.005	5	100	--	100	50	50	--
composition 153	1200	80	100	10	0.005	5	100	--	100	50	50	5

Preferred are the following compositions (table 13) comprising:

	FVIII [IU/ml]	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Tween®80 [% w/w]	Sucrose [% w/w]	Arginine [mM]	Isoleucine [mM]	Glutamate [mM]	Phenylalanine [mM]	Methionine [mM]	Glutathione [mM]
composition 154	1500	80	100	10	0.005	5	25	25	--	--	--	--
composition 155	1500	80	100	10	0.005	5	25	25	--	--	50	--
composition 156	1500	80	100	10	0.005	5	25	--	25	25	--	--
composition 157	1500	80	100	10	0.005	5	25	--	25	25	50	--
composition 158	1500	80	100	10	0.005	5	25	25	--	--	--	5
composition 159	1500	80	100	10	0.005	5	25	--	25	25	50	5
composition 160	1500	80	100	10	0.005	5	100	--	100	50	50	--
composition 161	1500	80	100	10	0.005	5	100	--	100	50	50	5

Preferred are the following compositions (table 14) comprising:

	FVIII [IU/ml]	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Tween®80 [% w/w]	Sucrose [% w/w]	Arginine [mM]	Isoleucine [mM]	Glutamate [mM]	Phenylalanine [mM]	Methionine [mM]	Glutathione [mM]
composition 162	2500	80	100	10	0.005	5	25	25	--	--	--	--
composition 163	2500	80	100	10	0.005	5	25	25	--	--	50	--
composition 164	2500	80	100	10	0.005	5	25	--	25	25	--	--
composition 165	2500	80	100	10	0.005	5	25	--	25	25	50	--
composition 166	2500	80	100	10	0.005	5	25	25	--	--	--	5
composition 167	2500	80	100	10	0.005	5	25	--	25	25	50	5
composition 168	2500	80	100	10	0.005	5	100	--	100	50	50	--
composition 169	2500	80	100	10	0.005	5	100	--	100	50	50	5

Preferred are the following compositions (table 15) comprising:

	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Sucrose [% w/w]
composition 170	40 - 195	at least 20	at least 8	2 - 10
composition 171	40 - 195	at least 20	at least 8	3 - 8
composition 172	40 - 195	at least 20	at least 8	4 - 6
composition 173	40 - 195	at least 20	at least 8	5
composition 174	40 - 195	at least 20	at least 10	2 - 10
composition 175	40 - 195	at least 20	at least 10	3 - 8
composition 176	40 - 195	at least 20	at least 10	4 - 6
composition 177	40 - 195	at least 20	at least 10	5
composition 178	40 - 195	at least 20	8 - 20	2 - 10
composition 179	40 - 195	at least 20	8 - 20	3 - 8
composition 180	40 - 195	at least 20	8 - 20	4 - 6
composition 181	40 - 195	at least 20	8 - 20	5
composition 182	40 - 195	at least 20	8 - 50	2 - 10
composition 183	40 - 195	at least 20	8 - 50	3 - 8
composition 184	40 - 195	at least 20	8 - 50	4 - 6
composition 185	40 - 195	at least 20	8 - 50	5
composition 186	40 - 195	at least 50	at least 6.5	2 - 10
composition 187	40 - 195	at least 50	at least 6.5	3 - 8
composition 188	40 - 195	at least 50	at least 6.5	4 - 6
composition 189	40 - 195	at least 50	at least 6.5	5
composition 190	40 - 195	at least 50	at least 10	2 - 10
composition 191	40 - 195	at least 50	at least 10	3 - 8
composition 192	40 - 195	at least 50	at least 10	4 - 6
composition 193	40 - 195	at least 50	at least 10	5
composition 194	40 - 195	at least 50	6.5 - 20	2 - 10
composition 195	40 - 195	at least 50	6.5 - 20	3 - 8
composition 196	40 - 195	at least 50	6.5 - 20	4 - 6
composition 197	40 - 195	at least 50	6.5 - 20	5
composition 198	40 - 195	at least 50	6.5 - 50	2 - 10
composition 199	40 - 195	at least 50	6.5 - 50	3 - 8
composition 200	40 - 195	at least 50	6.5 - 50	4 - 6
composition 201	40 - 195	at least 50	6.5 - 50	5
composition 202	40 - 195	50 - 100	at least 6.5	2 - 10
composition 203	40 - 195	50 - 100	at least 6.5	3 - 8
composition 204	40 - 195	50 - 100	at least 6.5	4 - 6
composition 205	40 - 195	50 - 100	at least 6.5	5
composition 206	40 - 195	50 - 100	at least 10	2 - 10
composition 207	40 - 195	50 - 100	at least 10	3 - 8
composition 208	40 - 195	50 - 100	at least 10	4 - 6
composition 209	40 - 195	50 - 100	at least 10	5

	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Sucrose [% w/w]
composition 210	40 - 195	50 - 100	6.5 - 20	2 - 10
composition 211	40 - 195	50 - 100	6.5 - 20	3 - 8
composition 212	40 - 195	50 - 100	6.5 - 20	4 - 6
composition 213	40 - 195	50 - 100	6.5 - 20	5
composition 214	40 - 195	50 - 100	6.5 - 50	2 - 10
composition 215	40 - 195	50 - 100	6.5 - 50	3 - 8
composition 216	40 - 195	50 - 100	6.5 - 50	4 - 6
composition 217	40 - 195	50 - 100	6.5 - 50	5
composition 218	40 - 195	100	at least 4	2 - 10
composition 219	40 - 195	100	at least 4	3 - 8
composition 220	40 - 195	100	at least 4	4 - 6
composition 221	40 - 195	100	at least 4	5
composition 222	40 - 195	100	at least 10	2 - 10
composition 223	40 - 195	100	at least 10	3 - 8
composition 224	40 - 195	100	at least 10	4 - 6
composition 225	40 - 195	100	at least 10	5
composition 226	40 - 195	100	4 - 20	2 - 10
composition 227	40 - 195	100	4 - 20	3 - 8
composition 228	40 - 195	100	4 - 20	4 - 6
composition 229	40 - 195	100	4 - 20	5
composition 230	40 - 195	100	4 - 50	2 - 10
composition 231	40 - 195	100	4 - 50	3 - 8
composition 232	40 - 195	100	4 - 50	4 - 6
composition 233	40 - 195	100	4 - 50	5

In another embodiment the compositions 170-233 further comprise a surfactant, e.g. Polyoxyethylene (20) sorbitan monooleate [e.g. Tween[®] 80], preferably at a concentration of at least 0.001 % w/w.

5

In another embodiment the compositions 170-233 further comprise one or more amino acids other than histidine. The other amino acid is selected from the list consisting of arginine, asparagine, aspartic acid, glutamic acid, glutamine, lysine, methionine, phenylalanine, isoleucine or mixtures thereof, preferably at a concentration of at least 1 mM.

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In another embodiment the compositions 170-233 further comprise an antioxidant, preferably at a concentration at least 0.05 mM. Examples of antioxidants include, but are not limited to reduced glutathione, methionine, cysteine, sodium sulfite, vitamin A, vitamin E, ascorbic acid, sodium ascorbate and mixtures thereof. In one embodiment of the invention the antioxidant

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is methionine, e.g., L- methionine.

In another embodiment, the compositions 170-233 have a pH of 6.0-8.0 or 6.5-7.5 or 6.8-7.2 or 7.

In another embodiment the compositions 170-233 further comprise

- 5 a. Polyoxyethylene (20) sorbitan monooleate [e.g. Tween® 80] as a surfactant, preferably at a concentration of at least 0.001 % w/w,
- b. one or more amino acids other than histidine selected from the list consisting of arginine, asparagine, aspartic acid, glutamic acid, glutamine, lysine, methionine, phenylalanine, isoleucine or mixtures thereof, preferably at a concentration of at least
10 1 mM,
- c. an antioxidant selected from the list consisting of reduced glutathione, methionine, cysteine, sodium sulfite, vitamin A, vitamin E, ascorbic acid, sodium ascorbate and mixtures thereof, preferably at a concentration of at least 0.05 mM and
- d. the compositions have a pH of 6.0-8.0 or 6.5-7.5 or 6.8-7.2 or 7.

15

Preferred are the following compositions (table 16) comprising:

	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Sucrose [% w/w]
composition 234	50 - 95	at least 20	at least 8	2 - 10
composition 235	50 - 95	at least 20	at least 8	3 - 8
composition 236	50 - 95	at least 20	at least 8	4 - 6
composition 237	50 - 95	at least 20	at least 8	5
composition 238	50 - 95	at least 20	at least 10	2 - 10
composition 239	50 - 95	at least 20	at least 10	3 - 8
composition 240	50 - 95	at least 20	at least 10	4 - 6
composition 241	50 - 95	at least 20	at least 10	5
composition 242	50 - 95	at least 20	8 - 20	2 - 10
composition 243	50 - 95	at least 20	8 - 20	3 - 8
composition 244	50 - 95	at least 20	8 - 20	4 - 6
composition 245	50 - 95	at least 20	8 - 20	5

	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Sucrose [% w/w]
composition 246	50 - 95	at least 20	8 - 50	2 - 10
composition 247	50 - 95	at least 20	8 - 50	3 - 8
composition 248	50 - 95	at least 20	8 - 50	4 - 6
composition 249	50 - 95	at least 20	8 - 50	5
composition 250	50 - 95	at least 50	at least 6.5	2 - 10
composition 251	50 - 95	at least 50	at least 6.5	3 - 8
composition 252	50 - 95	at least 50	at least 6.5	4 - 6
composition 253	50 - 95	at least 50	at least 6.5	5
composition 254	50 - 95	at least 50	at least 10	2 - 10
composition 255	50 - 95	at least 50	at least 10	3 - 8
composition 256	50 - 95	at least 50	at least 10	4 - 6
composition 257	50 - 95	at least 50	at least 10	5
compositon 258	50 - 95	at least 50	6.5 - 20	2 - 10
compositon 259	50 - 95	at least 50	6.5 - 20	3 - 8
compositon 260	50 - 95	at least 50	6.5 - 20	4 - 6
compositon 261	50 - 95	at least 50	6.5 - 20	5
compositon 262	50 - 95	at least 50	6.5 - 50	2 - 10
compositon 263	50 - 95	at least 50	6.5 - 50	3 - 8
compositon 264	50 - 95	at least 50	6.5 - 50	4 - 6
compositon 265	50 - 95	at least 50	6.5 - 50	5
compositon 266	50 - 95	50 - 100	at least 6.5	2 - 10
compositon 267	50 - 95	50 - 100	at least 6.5	3 - 8
compositon 268	50 - 95	50 - 100	at least 6.5	4 - 6
compositon 269	50 - 95	50 - 100	at least 6.5	5
compositon 270	50 - 95	50 - 100	at least 10	2 - 10
compositon 271	50 - 95	50 - 100	at least 10	3 - 8
compositon 272	50 - 95	50 - 100	at least 10	4 - 6
compositon 273	50 - 95	50 - 100	at least 10	5
compositon 274	50 - 95	50 - 100	6.5 - 20	2 - 10
compositon 275	50 - 95	50 - 100	6.5 - 20	3 - 8
compositon 276	50 - 95	50 - 100	6.5 - 20	4 - 6
compositon 277	50 - 95	50 - 100	6.5 - 20	5
compositon 278	50 - 95	50 - 100	6.5 - 50	2 - 10
compositon 279	50 - 95	50 - 100	6.5 - 50	3 - 8
compositon 280	50 - 95	50 - 100	6.5 - 50	4 - 6
compositon 281	50 - 95	50 - 100	6.5 - 50	5
compositon 282	50 - 95	100	at least 4	2 - 10
compositon 283	50 - 95	100	at least 4	3 - 8
compositon 284	50 - 95	100	at least 4	4 - 6
compositon 285	50 - 95	100	at least 4	5

	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Sucrose [% w/w]
compositon 286	50 - 95	100	at least 10	2 - 10
compositon 287	50 - 95	100	at least 10	3 - 8
compositon 288	50 - 95	100	at least 10	4 - 6
compositon 289	50 - 95	100	at least 10	5
compositon 290	50 - 95	100	4 - 20	2 - 10
compositon 291	50 - 95	100	4 - 20	3 - 8
compositon 292	50 - 95	100	4 - 20	4 - 6
compositon 293	50 - 95	100	4 - 20	5
compositon 294	50 - 95	100	4 - 50	2 - 10
compositon 295	50 - 95	100	4 - 50	3 - 8
compositon 296	50 - 95	100	4 - 50	4 - 6
compositon 297	50 - 95	100	4 - 50	5

In another embodiment the compositions 234-297 further comprise a surfactant, e.g. Polyoxyethylene (20) sorbitan monooleate [e.g. Tween[®] 80], preferably at a concentration of at least 0.001 % w/w.

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In another embodiment the compositions 234-297 further comprise one or more amino acids other than histidine. The other amino acid is selected from the list consisting of arginine, asparagine, aspartic acid, glutamic acid, glutamine, lysine, methionine, phenylalanine, isoleucine or mixtures thereof, preferably at a concentration of at least 1 mM.

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In another embodiment the compositions 234-297 further comprise an antioxidant, preferably at a concentration at least 0.05 mM. Examples of antioxidants include, but are not limited to reduced glutathione, methionine, cysteine, sodium sulfite, vitamin A, vitamin E, ascorbic acid, sodium ascorbate and mixtures thereof. In one embodiment of the invention the antioxidant is methionine, e.g., L- methionine.

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In another embodiment, the compositions 234-297 have a pH of 6.0-8.0 or 6.5-7.5 or 6.8-7.2 or 7.

20 In another embodiment the compositions 234-297 further comprise

e. Polyoxyethylene (20) sorbitan monooleate [e.g. Tween[®] 80] as a surfactant, preferably at a concentration of at least 0.001 % w/w,

f. one or more amino acids other than histidine selected from the list consisting of arginine, asparagine, aspartic acid, glutamic acid, glutamine, lysine, methionine, phenylalanine, isoleucine or mixtures thereof, preferably at a concentration of at least 1 mM,

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- g. an antioxidant selected from the list consisting of reduced glutathione, methionine, cysteine, sodium sulfite, vitamin A, vitamin E, ascorbic acid, sodium ascorbate and mixtures thereof, preferably at a concentration of at least 0.05 mM and
- h. the compositions have a pH of 6.0-8.0 or 6.5-7.5 or 6.8-7.2 or 7.

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Preferred are the following compositions (table 17) comprising:

	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Sucrose [% w/w]
compositon 298	60-75, e.g.65	at least 20	at least 8	2 - 10
compositon 299	60-75, e.g.65	at least 20	at least 8	3 - 8
compositon 300	60-75, e.g.65	at least 20	at least 8	4 - 6
compositon 301	60-75, e.g.65	at least 20	at least 8	5
compositon 302	60-75, e.g.65	at least 20	at least 10	2 - 10
compositon 303	60-75, e.g.65	at least 20	at least 10	3 - 8
compositon 304	60-75, e.g.65	at least 20	at least 10	4 - 6
compositon 305	60-75, e.g.65	at least 20	at least 10	5
compositon 306	60-75, e.g.65	at least 20	8 - 20	2 - 10
compositon 307	60-75, e.g.65	at least 20	8 - 20	3 - 8
compositon 308	60-75, e.g.65	at least 20	8 - 20	4 - 6
compositon 309	60-75, e.g.65	at least 20	8 - 20	5
compositon 310	60-75, e.g.65	at least 20	8 - 50	2 - 10
compositon 311	60-75, e.g.65	at least 20	8 - 50	3 - 8
compositon 312	60-75, e.g.65	at least 20	8 - 50	4 - 6
compositon 313	60-75, e.g.65	at least 20	8 - 50	5
compositon 314	60-75, e.g.65	at least 50	at least 6.5	2 - 10
compositon 315	60-75, e.g.65	at least 50	at least 6.5	3 - 8
compositon 316	60-75, e.g.65	at least 50	at least 6.5	4 - 6
compositon 317	60-75, e.g.65	at least 50	at least 6.5	5
compositon 318	60-75, e.g.65	at least 50	at least 10	2 - 10
compositon 319	60-75, e.g.65	at least 50	at least 10	3 - 8
compositon 320	60-75, e.g.65	at least 50	at least 10	4 - 6
compositon 321	60-75, e.g.65	at least 50	at least 10	5

	NaCl [mM]	Histidine [mM]	CaCl ₂ [mM]	Sucrose [% w/w]
compositon 322	60-75, e.g.65	at least 50	6.5 - 20	2 - 10
compositon 323	60-75, e.g.65	at least 50	6.5 - 20	3 - 8
compositon 324	60-75, e.g.65	at least 50	6.5 - 20	4 - 6
compositon 325	60-75, e.g.65	at least 50	6.5 - 20	5
compositon 326	60-75, e.g.65	at least 50	6.5 - 50	2 - 10
compositon 327	60-75, e.g.65	at least 50	6.5 - 50	3 - 8
compositon 328	60-75, e.g.65	at least 50	6.5 - 50	4 - 6
compositon 329	60-75, e.g.65	at least 50	6.5 - 50	5
compositon 330	60-75, e.g.65	50 - 100	at least 6.5	2 - 10
compositon 331	60-75, e.g.65	50 - 100	at least 6.5	3 - 8
compositon 332	60-75, e.g.65	50 - 100	at least 6.5	4 - 6
compositon 333	60-75, e.g.65	50 - 100	at least 6.5	5
compositon 334	60-75, e.g.65	50 - 100	at least 10	2 - 10
compositon 335	60-75, e.g.65	50 - 100	at least 10	3 - 8
compositon 336	60-75, e.g.65	50 - 100	at least 10	4 - 6
compositon 337	60-75, e.g.65	50 - 100	at least 10	5
compositon 338	60-75, e.g.65	50 - 100	6.5 - 20	2 - 10
compositon 339	60-75, e.g.65	50 - 100	6.5 - 20	3 - 8
compositon 340	60-75, e.g.65	50 - 100	6.5 - 20	4 - 6
compositon 341	60-75, e.g.65	50 - 100	6.5 - 20	5
compositon 342	60-75, e.g.65	50 - 100	6.5 - 50	2 - 10
compositon 343	60-75, e.g.65	50 - 100	6.5 - 50	3 - 8
compositon 344	60-75, e.g.65	50 - 100	6.5 - 50	4 - 6
compositon 345	60-75, e.g.65	50 - 100	6.5 - 50	5
compositon 346	60-75, e.g.65	100	at least 6.5	2 - 10
compositon 347	60-75, e.g.65	100	at least 6.5	3 - 8
compositon 348	60-75, e.g.65	100	at least 6.5	4 - 6
compositon 349	60-75, e.g.65	100	at least 6.5	5
compositon 350	60-75, e.g.65	100	at least 10	2 - 10
compositon 351	60-75, e.g.65	100	at least 10	3 - 8
compositon 352	60-75, e.g.65	100	at least 10	4 - 6
compositon 353	60-75, e.g.65	100	at least 10	5
compositon 354	60-75, e.g.65	100	6.5 - 20	2 - 10
compositon 355	60-75, e.g.65	100	6.5 - 20	3 - 8
compositon 356	60-75, e.g.65	100	6.5 - 20	4 - 6
compositon 357	60-75, e.g.65	100	6.5 - 20	5
compositon 358	60-75, e.g.65	100	6.5 - 50	2 - 10
compositon 359	60-75, e.g.65	100	6.5 - 50	3 - 8
compositon 360	60-75, e.g.65	100	6.5 - 50	4 - 6
compositon 361	60-75, e.g.65	100	6.5 - 50	5

In another embodiment the compositions 298-361 further comprise a surfactant, e.g. Polyoxyethylene (20) sorbitan monooleate [e.g. Tween[®] 80], preferably at a concentration of at least 0.001 % w/w.

- 5 In another embodiment the compositions 298-361 further comprise one or more amino acids other than histidine. The other amino acid is selected from the list consisting of arginine, asparagine, aspartic acid, glutamic acid, glutamine, lysine, methionine, phenylalanine, isoleucine or mixtures thereof, preferably at a concentration of at least 1 mM.
- 10 In another embodiment the compositions 298-361 further comprise an antioxidant, preferably at a concentration at least 0.05 mM. Examples of antioxidants include, but are not limited to reduced glutathione, methionine, cysteine, sodium sulfite, vitamin A, vitamin E, ascorbic acid, sodium ascorbate and mixtures thereof. In one embodiment of the invention the antioxidant is methionine, e.g., L- methionine.
- 15 In another embodiment, the compositions 298-361 have a pH of 6.0-8.0 or 6.5-7.5 or 6.8-7.2 or 7.
- In another embodiment the compositions 298-361 further comprise
- 20 i. Polyoxyethylene (20) sorbitan monooleate [e.g. Tween[®] 80] as a surfactant, preferably at a concentration of at least 0.001 % w/w,
- j. one or more amino acids other than histidine selected from the list consisting of arginine, asparagine, aspartic acid, glutamic acid, glutamine, lysine, methionine, phenylalanine, isoleucine or mixtures thereof, preferably at a concentration of at least
- 25 1 mM,
- k. an antioxidant selected from the list consisting of reduced glutathione, methionine, cysteine, sodium sulfite, vitamin A, vitamin E, ascorbic acid, sodium ascorbate and mixtures thereof, preferably at a concentration of at least 0.05 mM and
- l. the compositions have a pH of 6.0-8.0 or 6.5-7.5 or 6.8-7.2 or 7.

30

In a specific embodiment the invention relates to a composition obtainable by lyophilizing the aqueous composition described above. In another embodiment, the invention relates to a composition obtained by lyophilizing the aqueous composition described above.

- 35 In a specific embodiment the invention relates to a composition obtainable by reconstituting the lyophilized aqueous composition as described above with a suitable solvent.

In a specific embodiment a composition is obtainable by reconstituting the lyophilized aqueous composition as described above with a suitable solvent, wherein the suitable solvent is an aqueous solution.

5 In a specific embodiment a composition is obtainable by reconstituting the lyophilized aqueous composition as described above with an aqueous solution, wherein the aqueous solution is selected from the group comprising Ringer's solution, Ringer's lactate solution, and any other suitable solution for parenteral application.

10 In a specific embodiment a composition is obtainable by reconstituting the lyophilized aqueous composition as described above with an aqueous solution, wherein the aqueous solution is water, preferably "water for injection".

In a specific embodiment a composition is obtainable by reconstituting the lyophilized aqueous composition as described above with an aqueous solution, wherein the aqueous
15 solution is a sodium chloride solution.

In a specific embodiment a composition is obtainable by reconstituting the lyophilized aqueous composition as described above with an aqueous solution, wherein the aqueous
20 solution is a histidine solution.

Another aspect of the invention is the use of a composition described herein in the treatment of a blood coagulation disorder, e.g. hemophilia A.

25 In a specific embodiment of the compositions provided above, the composition is formulated for subcutaneous and/or intramuscular administration.

The compositions are administered orally, topically, transdermally, parenterally, by inhalation spray, vaginally, rectally or by intracranial injection, preferably parenterally. The term
30 parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intracisternal injection, or infusion techniques. Administration by intravenous, intradermal, intramuscular, intramammary, intraperitoneal, intrathecal, retrobulbar, intrapulmonary injection and/or surgical implantation at a particular site is contemplated as well. Generally, compositions are essentially free of pyrogens (see e.g. Ph. Eur. 7.0/2.06.08.00), as well as
35 other impurities that could be harmful to the recipient.

EXAMPLES

The following examples were carried out using a FVIII molecule (SEQ ID NO:2; construct dBN(64-53) described in WO 2004/067566). This FVIII molecule will be referred to as "CSL627" in the following.

5

Chromogenic FVIII:C Assay

The chromogenic FVIII:C assay was performed using the Coamatic FVIII test kit (Chromogenix-Instrumentation Laboratory SpA V. le Monza 338 - 20128 Milano, Italy).

- 10 Principle of the assay: In the presence of calcium and phospholipid, Factor X is activated by Factor IXa to Factor Xa. This reaction is stimulated by Factor VIIIa as cofactor. F VIIIa is formed by low amounts of thrombin in the reaction mixture from F VIII in the sample to be measured. When using the optimum concentrations of Ca^{2+} , phospholipid and Factor IXa and an excess quantity of Factor X, activation of Factor X is proportional to the potency of Factor
- 15 VIII. Activated Factor X releases the chromophore pNA from the chromogenic substrate S-2765. The release of pNA, measured at 405 nm, is therefore proportional to the amount of FXa formed and, therefore, also to the Factor VIII activity of the sample. The assay was adapted to be performed on automated coagulation analyzers, either the Behring Coagulation Timer (BCT) or Behring Coagulation System (BCS), both from Siemens
- 20 Healthcare Diagnostics GmbH, Ludwig-Erhard-Straße 12, 65760 Eschborn, Germany.

One stage coagulation assay

- The FVIII:C one stage coagulation assay was performed using the Pathromtin SL reagent and FVIII deficient plasma, both from Siemens Healthcare Diagnostics products GmbH, Emil-
- 25 von-Behring –Str. 76, 35041 Marburg, Germany.

- Principle of the assay: The test is executed as a modified version of the activated Partial Thromboplastin Time (aPTT)- assay: Incubation of plasma with phospholipids and a surface activator leads to the activation of factors of the intrinsic coagulation system. Addition of
- 30 calcium ions triggers the coagulation cascade. The time to formation of a measurable fibrin clot is measured. The assay is executed in the presence of Factor VIII deficient plasma. The coagulation capability of the deficient plasma is restored by Coagulation Factor VIII included in the sample to be tested. The shortening of coagulation time is proportional to the amount of Factor VIII present in the sample. The activity of Coagulation Factor VIII is quantified by
- 35 direct comparison to a standard preparation with a known activity of Factor VIII in International Units.

The assay was adapted to be performed on automated coagulation analyzers, either the Behring Coagulation Timer (BCT) or Behring Coagulation System (BCS), both from Siemens Healthcare Diagnostics GmbH, Ludwig-Erhard-Straße 12, 65760 Eschborn, Germany.

5 *High Molecular Weight Components (HMWC) by Size exclusion HPLC*

The size exclusion HPLC was performed using a COSMOSIL Diol-300-II 7.5 x 600 mm column (Nacalai Tesque, Kyoto, Japan) and fluorescence detection at excitation wavelength 280 nm and emission wavelength 340 nm. The composition of the mobile phase was 300 mM NaCl, 20 mM HEPES, 10 mM CaCl₂ * 2H₂O, 0,005 % Tween® 80, 10 % Isopropanol, pH 7.0. Elution was isocratic at a flow rate of 0.5 mL/min at ambient room temperature over 75 minutes.

Example 1:

Preparation of FVIII formulations and evaluation of the solution properties following buffer exchange via desalting columns

Purified CSL627 at a FVIII:C activity (chromogenic substrate method) of 9500 IU/ml was formulated into the desired compositions by buffer exchange via NAP-25 desalting columns (GE Healthcare Sephadex™ G 25; Cat. No. 17-0852-01) according to the suppliers instructions. This buffer exchange resulted in a dilution factor of 1.4.

The different compositions were then investigated for their appearance (turbidity) and yield in FVIII:C activity.

The yield of FVIII:C was calculated as the percentage of the amount of FVIII:C in the obtained composition following buffer exchange divided by the amount of FVIII:C in the solution prior to buffer exchange with appropriate adjustment for dilution by the buffer exchange (dilution factor 1.4).

Table 18.

Formulation no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	Tween® 80 [% v/v]	turbid yes/no	FVIII:C Yield * [%]
01-001	80	5	100	10	0.005	no	112
01-002 (comparative)	10	5	100	10	0.005	yes	48
01-003 (comparative)	30	5	100	10	0.005	yes	98

* FVIII:C activity yield determined via a chromogenic substrate activity assay following buffer exchange via desalting columns

Example 2:

Preparation of FVIII formulations and evaluation of the solution properties following buffer exchange via desalting columns

- 5 The different compositions were obtained by buffer exchange as described in example 1 (FVIII:C activity in the starting material was 9733 IU/mL). The compositions were evaluated for their appearance following buffer exchange. In addition the compositions were then sterile filtered (0.22 μ m) and assayed for their FVIII:C activity directly upon filtration (time 0) and following storage at +2 – +8 °C for 1, 2, 3, 15, 30, and 60 days and storage at +25 °C and
10 +40 °C for 1, 2 and 3 days.

The recovery (stability) was calculated as the percentage of FVIII:C (Chromogenic substrate FVIII activity assay) after storage divided by the FVIII:C activity at Time 0. The FVIII:C activity at Time 0 (after 0.22 μ m filtration) was defined as 100%.

Table 19

							Stability: FVIII:C Coamatic Recovery * [%]						
							+2 to +8 °C						
Form. no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	Tween® 80 [% v/v]	turbid yes/no	Time 0	1 day	2 days	3 days	15 days	30 days	60 days
02-001	40	5	100	10	0.005	no	100	103	107	100	97	97	93
02-002	50	5	100	10	0.005	no	100	96	104	97	92	90	81
02-003	60	5	100	10	0.005	no	100	102	111	114	105	98	85
02-004	70	5	100	10	0.005	no	100	110	98	104	93	94	87
02-005	80	5	100	10	0.005	no	100	100	106	102	100	93	91
02-006	100	5	100	10	0.005	no	100	100	111	106	101	96	87
02-007	150	5	100	10	0.005	no	100	98	109	110	92	88	85

* FVIII:C recovery based on FVIII:C activity measurements (Chromogenic substrate activity assay) following storage at different temperature conditions (stability test in liquid state)

Table 20

							Stability: FVIII:C Coamatic Recovery * [%]			
							+25 °C			
Form.no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	Tween® 80 [% v/v]	turbid yes/no	Time 0	1 day	2 days	3 days
02-001	40	5	100	10	0.005	no	100	104	105	103
02-002	50	5	100	10	0.005	no	100	95	95	102
02-003	60	5	100	10	0.005	no	100	105	104	116
02-004	70	5	100	10	0.005	no	100	104	96	100
02-005	80	5	100	10	0.005	no	100	99	103	96
02-006	100	5	100	10	0.005	no	100	107	102	105
02-007	150	5	100	10	0.005	no	100	106	103	103

* FVIII:C recovery based on FVIII:C activity measurements (Chromogenic substrate activity assay) following storage at different temperature conditions (stability test in liquid state)

Table 21

							Stability: FVIII:C Coamatic Recovery * [%]			
							+40 °C			
Form.no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	Tween® 80 [% v/v]	turbid yes/no	Time 0	1 day	2 days	3 days
02-001	40	5	100	10	0.005	no	100	103	93	101
02-002	50	5	100	10	0.005	no	100	91	89	93
02-003	60	5	100	10	0.005	no	100	102	105	104
02-004	70	5	100	10	0.005	no	100	96	97	97
02-005	80	5	100	10	0.005	no	100	107	91	89
02-006	100	5	100	10	0.005	no	100	105	95	93
02-007	150	5	100	10	0.005	no	100	105	104	103

* FVIII:C recovery based on FVIII:C activity measurements (Chromogenic substrate activity assay) following storage at different temperature conditions (stability test in liquid state)

Example 3:

Appearance of the solution following buffer exchange via desalting columns

- 5 The different compositions were obtained by buffer exchange as described in example 1 (FVIII:C activity by chromogenic substrate FVIII activity assay in the starting material was 8317 IU/mL). The compositions were evaluated for their appearance following buffer exchange.

10 Table 22

Form.no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	Tween® 80 [% v/v]	turbid yes/no
03-001 (comparative)	0	5	100	10	0.005	yes
03-002 (comparative)	10	5	100	10	0.005	yes
03-003 (comparative)	20	5	100	10	0.005	yes
03-004 (comparative)	30	5	100	10	0.005	yes
03-005	40	5	100	10	0.005	no
03-006	50	5	100	10	0.005	no
03-007	80	5	100	10	0.005	no
03-008	80	0.5	100	10	0.005	no
03-009	80	5	20	10	0.005	no
03-010 (comparative)	80	5	100	3	0.005	yes
03-011 (comparative)	80	0.5	20	3	0.005	yes
03-012	80	0.5	20	10	0.005	no
03-013 (comparative)	80	0.5	100	3	0.005	yes
03-014 (comparative)	80	5	20	3	0.005	yes

Example 4:

Yield in FVIII:C activity and appearance of the solution following buffer exchange via desalting columns and recovery of FVIII:C activity upon storage.

5

The different compositions were obtained by buffer exchange as described in example 1 (FVIII:C activity in the starting material was 4780 IU/mL).

10 The compositions were evaluated for their appearance following buffer exchange. In addition the compositions were then sterile filtered (0.22 μ m) and assayed for their FVIII:C activity (Chromogenic substrate assay) directly upon filtration (time 0) and following storage at 2 – 8 °C for 1,2 and 3 days and storage at 25 °C and 40 °C for 3 days.

15 The yield of the formulation process in FVIII:C activity was calculated as the percentage of FVIII:C in the obtained composition following buffer exchange divided by the FVIII:C in the solution prior to buffer exchange with appropriate adjustment for dilutions. The recovery (stability) upon storage was calculated as the percentage of FVIII:C after storage divided by the FVIII:C activity at Time 0. The FVIII:C activity at Time 0 (after 0.22 μ m filtration) was defined as 100%.

Table 23

								Stability: FVIII:C Coamatic Recovery ** [%]					
									+2 to +8 °C			+25 °C	+40 °C
Form.no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	Tween® 80 [% v/v]	turbid yes/no	FVIII:C Coamatic Yield * [%]	Time 0	1 days	2 days	3 days	3 days	3 days
04-001	80	5	100	10	0.005	no	99	100	n/a	n/a	88	88	81
04-002 (comparative)	50	5	100	1	0.005	yes	76	100	n/a	n/a	88	72	74
04-003	50	5	100	7	0.005	no	95	100	n/a	n/a	90	95	81
04-004	50	5	100	10	0.005	no	98	100	n/a	n/a	93	93	80
04-005	50	5	100	15	0.005	no	93	100	n/a	n/a	92	94	83
04-006	50	5	100	20	0.005	no	94	100	n/a	n/a	92	96	81

* FVIII:C yield (based on FVIII activity measurements with a chromogenic substrate FVIII activity assay) following buffer exchange via desalting columns

** FVIII:C recovery (based on FVIII activity measurements with a chromogenic substrate FVIII activity assay) following storage at different temperature conditions (stability test in liquid state)

Example 5:

HMWC formation (SE-HPLC) in freeze-dried preparations upon storage at elevated temperature.

5

The FVIII:C activity and the concentration of the excipients of the formulations of this example were adjusted by diluting a FVIII concentrate with appropriate buffer solutions. The formulations obtained showed FVIII:C concentrations of 430 – 480 IU/mL. The solutions were then dispensed (2.5 mL) and freeze-dried. The obtained lyophilisates were stored at +40 °C and samples taken after 1, 3, 6 and 12 months. Samples were taken from the reconstituted solutions prior to and following storage of the lyophilisates and frozen at – 70 °C. HMWC were determined by SE-HPLC in the samples following thawing in a water bath (+37 °C).

10

Table 24

						Storage stability of lyophilisates: HMWC by SE-HPLC [%]				
							+40 °C			
Form.no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	Tween® 80 [% v/v]	after FD (Time 0)	1 month	3 month	6 month	12 month
05-001	52	5	100	10	0.004	0.76	1.39	0.41	0.25	0.73
05-002 (comparative)	280	0.6	20	3.4	0.02	0.56	2.89	3.16	6.13	5.75
05-003 (comparative)	340	2.55	52.5	5.5	0.05	0.30	2.07	2.18	2.79	4.17
05-004 (comparative)	340	2.55	52.5	0.64	0.05	0.34	2.24	2.15	2.97	4.48
05-005 (comparative)	340	2.55	3.61	5.5	0.05	0.31	1.84	2.19	3.50	5.69
05-006 (comparative)	600	5	3.61	0.64	0.1	0.38	3.38	3.86	7.21	11.43
05-007 (comparative)	600	5	3.61	10	0.004	1.44	1.81	1.81	5.48	7.73
05-008 (comparative)	600	5	100	0.64	0.004	1.03	1.64	2.18	4.90	6.71

Example 6:

FVIII:C chromogenic substrate activity assay and HMWC formation (SE-HPLC) in freeze-dried preparations upon storage at elevated temperature

5

The FVIII:C activity and the concentration of the excipients were adjusted by diluting a FVIII concentrate with appropriate buffer solutions. The solutions were then dispensed (2.5 mL) and freeze-dried. The obtained lyophilisates were stored at +40 °C and samples taken after 1, 3, 6, 12, 18 and 24 months. Samples were taken from the reconstituted solutions prior to and following storage of the lyophilisates and frozen at – 70 °C. HMWC were determined in the samples stored frozen following thawing in a water bath (+37 °C).

10

FVIII:C activity (Chromogenic substrate FVIII activity assay) was determined in the fresh solutions prior to freeze-drying and in the fresh reconstituted lyophilisates directly following freeze-drying or after storage. The recovery (stability) was calculated as the percentage of FVIII:C after storage divided by the FVIII:C activity at Time 0. The FVIII:C activity at Time 0 (After freeze-drying) was defined as 100%.

15

Table 25

								Storage stability of lyophilisates: FVIII:C Coamatic Recovery [%]							
								+40 °C							
Form. no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	Tween® 80 [% v/v]	dosage form [IU]	FVIII:C activity prior to freeze- drying [IU/ml]	after FD (Time 0)	1 month	3 month	6 month	9 month	12 month	18 month	24 month
06-001 (comparative)	280	0.6	20	3.4	0.02	500	219	100	94	84	86	64	58	50	46
06-002	80	5	100	10	0.005	500	218	100	96	96	98	91	81	89	100
06-003 (comparative)	280	0.6	20	3.4	0.02	1000	437	100	106	87	88	78	65	59	55
06-004	80	5	100	10	0.005	1000	428	100	103	102	110	96	86	87	93
06-005 (comparative)	280	0.6	20	3.4	0.02	3000	1440	100	91	83	74	69	55	57	42
06-006	80	5	100	10	0.005	3000	1368	100	84	89	94	86	n.a.	82	89

Table 26

								Storage stability of lyophilisates: HMWC by SE-HPLC [%]							
								+40 °C							
Form. no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	Tween® 80 [% v/v]	dosage form [IU]	FVIII:C activity prior to freeze- drying [IU/ml]	after FD (Time 0)	1 month	3 month	6 month	9 month	12 month	18 month	24 month
06-001 (comparative)	280	0.6	20	3.4	0.02	500	219	0.28	2.99	2.24	2.99	3.91	3.65	12.14	7.19
06-002	80	5	100	10	0.005	500	218	0.13	0.13	0.30	0.56	0.66	0.74	1.42	0.79
06-003 (comparative)	280	0.6	20	3.4	0.02	1000	437	0.59	1.94	5.00	4.26	3.29	3.91	6.88	7.13
06-004	80	5	100	10	0.005	1000	428	0.23	0.50	0.96	1.25	0.58	0.73	1.46	1.72
06-005 (comparative)	280	0.6	20	3.4	0.02	3000	1440	0.53	2.50	4.86	9.56	4.51	9.12	6.95	15.95
06-006	80	5	100	10	0.005	3000	1368	0.13	0.33	0.38	0.31	1.04	n.a.	1.02	2.03

Example 7:

Preparation of FVIII formulations and evaluation of the solution properties following buffer exchange via desalting columns.

5

Purified CSL627 was formulated into the desired compositions by buffer exchange via NAP-25 desalting columns (GE Healthcare Sephadex™ G 25; Cat. No. 17-0852-01) according to the suppliers instructions and as described in example 1. Subsequently, the different compositions were diluted based on the chromogenic activity assay to obtain a FVIII:C activity (potency) using a chromogenic substrate FVIII activity assay of about 7000 IU/mL. The different compositions were then investigated for their appearance (turbidity).

10

Table 27

Form. no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	GSH [mM]	Tween® 80 (%)	turbid yes/no
07-001	65	5.0	100	10	0.25	0.005	no
07-002	65	5.0	100	10	0.25	0.005	no
07-003	65	5.0	100	5.5	0.25	0.005	no
07-004 (comparative)	65	5.0	100	3.0	0.25	0.005	yes
07-005 (comparative)	65	5.0	100	1.0	0.25	0.005	yes
07-006	65	5.0	55	10	0.25	0.005	no
07-007 (comparative)	65	5.0	55	5.5	0.25	0.005	yes
07-008 (comparative)	65	5.0	55	5.5	0.25	0.005	yes
07-009 (comparative)	65	5.0	55	5.5	0.25	0.005	yes
07-010 (comparative)	65	5.0	55	3.0	0.25	0.005	yes
07-011 (comparative)	65	5.0	55	1.0	0.25	0.005	yes
07-012	65	5.0	30	10	0.25	0.005	no
07-013 (comparative)	65	5.0	30	5.5	0.25	0.005	yes
07-014 (comparative)	65	5.0	30	1.0	0.25	0.005	yes
07-015	65	5.0	10	10	0.25	0.005	no
07-016 (comparative)	65	5.0	10	5.5	0.25	0.005	yes
07-017 (comparative)	65	5.0	10	3.0	0.25	0.005	yes
07-018 (comparative)	65	5.0	10	1.0	0.25	0.005	yes

Results are shown graphically in Figure 1. Dots represent a turbidity above a threshold level of 18 NTU, diamonds represent a clear solution (turbidity less than or equal to 18 NTU).

5 **Example 8**

HMWC formation (SE-HPLC) in freeze-dried preparations upon storage at elevated temperature

- 10 The FVIII:C activity and the concentration of the excipients of the formulations of this example were adjusted by diluting a FVIII concentrate (CSL627) with appropriate buffer solutions. The formulations obtained showed FVIII:C concentrations of about 390 - 435 IU/mL. The solutions were then dispensed (2.5 mL) and freeze-dried. The obtained lyophilisates were stored at +40 °C and samples taken after 1, 3, 6, 9 and 12 months.
- 15 Samples were taken from the reconstituted solutions prior to and following storage of the lyophilisates and frozen at – 70 °C. HMWC were determined by SE-HPLC in the samples following thawing in a water bath (+37 °C).

Table 28

							Storage stability of lyophilisates: HMWC by SE-HPLC [%]					
								+40 °C				
Form.no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	GSH [mM]	Tween® 80 [% v/v]	after FD (Time 0)	1 month	3 month	6 month	9 month	12 month
08-001 (comparative)	500	5.0	100	10	0.25	0.005	1.27	1.27	2.22	3.11	2.92	4.77
08-002 (comparative)	300	5.0	100	10	0.25	0.005	0.29	0.58	1.06	1.86	2.04	1.92
08-003	150	5.0	100	10	0.25	0.005	0.47	0.42	0.68	0.74	0.81	0.56
08-004	100	5.0	100	10	0.25	0.005	0.32	0.40	0.74	0.65	0.72	0.62
08-005	65	5.0	100	15	0.25	0.005	0.34	0.34	0.84	0.77	0.96	0.75
08-006	65	5.0	100	10	0.25	0.005	0.34	0.42	0.77	0.67	0.87	0.72
08-007	65	5.0	100	7	0.25	0.005	0.53	0.51	0.78	0.79	1.10	0.87
08-008	65	5.0	100	5	0.25	0.005	0.49	0.53	0.93	0.83	1.17	0.84

Example 9

FVIII:C chromogenic substrate activity assay and HMWC formation (SE-HPLC) in freeze-dried preparations upon storage at elevated temperature

5

The FVIII:C activity and the concentration of the excipients were adjusted by diluting a FVIII concentrate (CSL627) with appropriate buffer solutions. The solutions were then dispensed (2.5 mL) and freeze-dried. The obtained lyophilisates were stored at +40 °C and samples taken after 1, 3, 6, 9 and 12 months. Samples were taken from the reconstituted solutions prior to and following storage of the lyophilisates and frozen at – 70 °C. HMWC were determined in the samples stored frozen following thawing in a water bath (+37 °C).

10

FVIII:C activity (Chromogenic substrate FVIII activity assay) was determined in the fresh solutions prior to freeze-drying and in the fresh reconstituted lyophilisates directly following freeze-drying or after storage. The recovery (stability) was calculated as the percentage of FVIII:C after storage divided by the FVIII:C activity at Time 0. The FVIII:C activity at Time 0 (After freeze-drying) was defined as 100%.

15

Table 29

									Storage stability of lyophilisates: FVIII:C Coamatic					
									Recovery [%]					
									+40 °C					
Form.no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	GSH [mM]	Tween® 80 [% v/v]	dosage form [IU]	FVIII:C activity prior to freeze- drying [IU/ml]	after FD (Time 0)	1 month	3 month	6 month	9 month	12 month
09-001	65	5.0	100	10	0.25	0.005	1000	417	100	102	100	96	100	106
09-002	65	5.0	100	10	0.25	0.005	1000	447	100	107	103	93	93	95
09-003	65	5.0	100	5.5	0.25	0.005	1000	411	100	98	104	90	93	91
09-004	65	5.0	55	10	0.25	0.005	1000	389	100	103	105	95	105	107
09-005	65	5.0	30	10	0.25	0.005	1000	383	100	108	100	97	98	102
09-006	65	5.0	10	10	0.25	0.005	1000	384	100	110	101	95	94	90

Table 30

									Storage stability of lyophilisates: HMWC by SE-HPLC [%] (n.t. = not tested)					
										+40 °C				
Form.no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	GSH [mM]	Tween® 80 [% v/v]	dosage form [IU]	FVIII:C activity prior to freeze- drying [IU/ml]	after FD (Time 0)	1 month	3 month	6 month	9 month	12 month
09-001	65	5.0	100	10	0.25	0.005	1000	417	0.19	n.t.	0,64	0,39	0,87	n.t.
09-002	65	5.0	100	10	0.25	0.005	1000	447	0.39	n.t.	0,57	0,46	0,64	n.t.
09-003	65	5.0	100	5.5	0.25	0.005	1000	411	1.10	n.t.	0,74	0,35	0,64	n.t.
09-004	65	5.0	55	10	0.25	0.005	1000	389	0.30	n.t.	1,00	0,76	1,10	n.t.
09-005	65	5.0	30	10	0.25	0.005	1000	383	0.26	n.t.	0,88	0,69	1,13	n.t.
09-006	65	5.0	10	10	0.25	0.005	1000	384	0.25	n.t.	0,98	1,03	1,26	n.t.

Example 10

FVIII:C chromogenic substrate activity assay and HMWC formation (SE-HPLC) in freeze-dried preparations upon storage at elevated temperature.

- 5 The FVIII:C activity and the concentration of the excipients were adjusted by diluting a FVIII concentrate (CSL627) with appropriate buffer solutions. The solutions were then dispensed (2.5 mL) and freeze-dried. The obtained lyophilisates were stored at +40 °C and samples taken after 3, 6 and 9 months. Samples were taken from the reconstituted solutions prior to and following storage of the lyophilisates and frozen at – 70 °C. HMWC were determined in
10 the samples stored frozen following thawing in a water bath (+37 °C).

- FVIII:C activity (Chromogenic substrate FVIII activity assay) was determined in the fresh solutions prior to freeze-drying and in the fresh reconstituted lyophilisates directly following freeze-drying or after storage. The recovery (stability) was calculated as the percentage of
15 FVIII:C after storage divided by the FVIII:C activity at Time 0. The FVIII:C activity at Time 0 (After freeze-drying) was defined as 100%.

Table 31

									Storage stability of lyophilisates: FVIII:C Coamatic Recovery [%]			
									+40 °C			
Form.no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	GSH [mM]	Tween® 80 [% v/v]	dosage form [IU]	FVIII:C activity prior to freeze-drying [IU/ml]	after FD (Time 0)	3 month	6 month	9 month
10-001	65	5.0	100	10	0.25	0.005	250	91	100	96	92	95
10-002	65	5.0	100	10	0.25	0.005	1000	402	100	99	88	96
10-003	65	5.0	100	10	0.25	0.005	3000	1340	100	90	85	87

Table 32

									Storage stability of lyophilisates: FVIII:C Coamatic Recovery [%]			
									+40 °C			
Form.no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	L-Methionine [mM]	Tween® 80 [% v/v]	dosage form [IU]	FVIII:C activity prior to freeze-drying [IU/ml]	after FD (Time 0)	3 month	6 month	9 month
10-004	65	5.0	100	10	5.0	0.005	250	96	100	90	90	92
10-005	65	5.0	100	10	5.0	0.005	1000	416	100	96	97	90
10-006	65	5.0	100	10	5.0	0.005	3000	1324	100	97	93	94

Table 33

									Storage stability of lyophilisates: HMWC by SE-HPLC [%]			
									+40 °C			
Form.no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	GSH [mM]	Tween® 80 [% v/v]	dosage form [IU]	FVIII:C activity prior to freeze-drying [IU/ml]	after FD (Time 0)	3 month	6 month	9 month
10-001	65	5.0	100	10	0.25	0.005	250	91	0,28	0,43	0,60	0,86
10-002	65	5.0	100	10	0.25	0.005	1000	402	0,36	0,39	1,01	1,05
10-003	65	5.0	100	10	0.25	0.005	3000	1340	0,55	0,55	1,00	1,08

Table 34

									Storage stability of lyophilisates: HMWC by SE-HPLC [%]			
									+40 °C			
Form.no #	NaCl [mM]	Sucrose [% w/v]	Histidine [mM]	CaCl ₂ [mM]	L-Methionine [mM]	Tween® 80 [% v/v]	dosage form [IU]	FVIII:C activity prior to freeze-drying [IU/ml]	after FD (Time 0)	3 month	6 month	9 month
10-004	65	5.0	100	10	5.0	0.005	250	96	0,33	0,23	0,59	0,56
10-005	65	5.0	100	10	5.0	0.005	1000	416	0,41	0,45	1,09	0,97
10-006	65	5.0	100	10	5.0	0.005	3000	1324	0,62	0,84	1,12	1,25

5 CLAIMS

1. An aqueous composition of coagulation Factor VIII, comprising:

- a. a FVIII molecule;
- b. 40 to 195 mM of a sodium salt;
- c. histidine;
- 10 d. at least 1 mM of a calcium salt; and
- e. a surfactant;

wherein $[\text{His}] \geq 180 \text{ mM} - 20 \cdot [\text{Ca}^{2+}]$, wherein $[\text{Ca}^{2+}]$ is the concentration of calcium ions in the aqueous composition in millimole per liter, and $[\text{His}]$ is the concentration of histidine in the aqueous composition in millimole per liter, with the proviso that $[\text{His}] > 0$; and wherein the
15 osmolality of the composition is 600 mOsmol/L or less.

2. The aqueous composition according to claim 1, wherein the concentration of the sodium salt in the aqueous composition is 45 to 95 mM.

20 3. The aqueous composition according to claim 1 and 2, wherein $[\text{His}]$ is 5 to 200 mM.

4. The aqueous composition according to any one of the preceding claims, wherein $[\text{Ca}^{2+}]$ is 5 to 100 mM.

25 5. The aqueous composition according to any one of the preceding claims, wherein the pH of the aqueous composition is from 5 to 9.

6. The aqueous composition according to any one of the preceding claims, wherein the composition, upon (i) lyophilization, (ii) storage of the lyophilized composition at a
30 temperature of +25°C and a relative humidity of 40% for a period of 12 months, and (iii) subsequent reconstitution of the lyophilized composition in distilled water, shows a recovery of FVIII:C activity of at least 80%, relative to the same composition upon (i) lyophilization and, without storage of the lyophilized composition, (ii) subsequent immediate reconstitution in distilled water.

35 7. The aqueous composition according to any one of the preceding claims, wherein the aqueous composition retains at least 80% of its Factor VIII activity for at least 48 hours, when stored in the liquid state at 4°C.

8. The aqueous composition according to any one of the preceding claims, wherein the calcium salt is calcium chloride.
9. The aqueous composition according to any one of the preceding claims, wherein the sodium salt is sodium chloride.
10. The aqueous composition according to any one of the preceding claims, wherein the composition further comprises a carbohydrate.
11. The aqueous composition according to claim 10, wherein the carbohydrate is sucrose.
12. The aqueous composition according to claim 10 or 11, wherein the concentration of the carbohydrate is 1 to 20 % (w/w).
13. The aqueous composition according to any one of the preceding claims, wherein the concentration of the surfactant is 0.001 to 0.2 % (v/v).
14. The aqueous composition according to any one of the preceding claims, wherein the surfactant is a non-naturally occurring surfactant.
15. The aqueous composition according to any one of the preceding claims, wherein the composition further comprises at least one amino acid other than histidine.
16. The aqueous composition according to claim 15, wherein said at least one amino acid other than histidine is selected from the group consisting of arginine, asparagine, aspartic acid, glutamic acid, glutamine, lysine, methionine, phenylalanine, leucine, isoleucine and combinations thereof.
17. The aqueous composition according to any one of the preceding claims, wherein the composition further comprises at least one antioxidant.
18. The aqueous composition according to claim 17, wherein said at least one antioxidant is selected from the group consisting of reduced glutathione, methionine, cysteine, sodium sulfite, vitamin A, vitamin E, ascorbic acid, sodium ascorbate and combinations thereof.

19. The aqueous composition according to claim 17 or 18, wherein the concentration of said at least one antioxidant is 0.05 – 100 mM.
20. The aqueous composition according to any one of the preceding claims, wherein the
5 FVIII molecule is a non-naturally occurring FVIII molecule, preferably a recombinantly produced FVIII molecule, e.g. (i) a B-domain deleted or truncated FVIII molecule, (ii) a recombinantly produced two-chain FVIII molecule, or (iii) a single-chain FVIII molecule.
21. A composition obtainable by lyophilizing the aqueous composition according to any
10 one of the preceding claims.
22. An aqueous composition of coagulation Factor VIII, obtainable by reconstituting the lyophilized composition according to claim 21 with an aqueous solution.
- 15 23. A method of stabilizing a FVIII molecule, comprising mixing the components defined in claim 1 to obtain an aqueous composition, and lyophilizing the aqueous composition.