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(54) Title: FACTOR VII AND VIIA COMPOSITIONS

(57) Abstract: The invention relates to stabilized compositions comprising a Factor VII or Factor VIIa polypeptide or a polypeptide variant thereof and a histidine residue as a buffering agent.



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FACTOR VII AND VIIA COMPOSITIONS

CROSS-REFERENCES TO RELATED APPLICATIONS

5 The present application claims priority to and benefit of the United States Provisional Patent Application Number 60/870,948, filed on December 20, 2006, the disclosure of which is incorporated herein by reference in its entirety for all purposes.

FIELD OF THE INVENTION

10 The present invention relates to storage-stable compositions of a Factor VII or Factor VIIa polypeptide or a polypeptide variant thereof.

BACKGROUND OF THE INVENTION

Factor VII (FVII), an important blood clotting protein in the blood coagulation
15 cascade, is a vitamin K-dependent plasma protein synthesized in the liver and secreted into the blood as a single-chain glycoprotein with a molecular weight of 50 kilodalton (kDa). The FVII zymogen is converted into an activated form, Factor VIIa (FVIIa), by proteolytic cleavage at a single site, R152-I153, resulting in two chains linked by a single disulfide bridge. Recombinant human FVIIa is commercially available from Novo Nordisk under the
20 name NovoSeven® and is used for the treatment of bleeding episodes, e.g., in hemophilia or trauma, facilitating blood coagulation/clotting. Recombinantly produced variants of human FVII or FVIIa have also been reported.

NovoSeven® is a freeze-dried FVIIa product which must be reconstituted before use. A vial (1.2 mg) of NovoSeven® contains 1.2 mg recombinant human FVIIa (rhFVIIa), 5.84
25 mg NaCl, 2.94 mg CaCl₂·2H₂O, 2.64 mg glycylglycine, 0.14 mg polysorbate 80 and 60.0 mg mannitol; it is reconstituted to pH 5.5 by 2.0 ml water for injection. When reconstituted, the protein is stable for use for 24 hours at 2-8°C.

While it is generally advantageous to be able to provide therapeutic proteins such as FVII/FVIIa in liquid form for ease of use, due to the reduced stability of proteins in liquid
30 form as opposed to dry form, such proteins are often provided in dry form, e.g., in lyophilized (freeze-dried) form, in order to obtain a sufficient long-term storage stability. The instability of proteins during storage can have various causes, including physical instability as a result of denaturation or aggregation, and chemical instability as a result of hydrolysis, deamidation or

oxidation. The consequences of this chemical and/or physical degradation can include not only a significant loss of FVII activity but also, e.g., an immunogenic response due to the presence of protein aggregates. Additionally, a special challenge is presented by proteins and peptides with autoproteolytic domains, as these tend to self-digest upon storage in a liquid dosage form.

Lyophilized formulations of FVII/FVIIa proteins are described, e.g., in WO 01/12653, WO 03/092731, WO 2004/000347 and WO 2005/058283. Although various compositions or formulations of FVII/FVIIa are known, there is still room for improvement.

SUMMARY OF THE INVENTION

In one aspect, the invention provides a lyophilized composition suitable for reconstitution with water, comprising a Factor VII or Factor VIIa polypeptide (e.g., rhFVII or rhFVIIa) and histidine as a buffering agent, wherein the concentration of histidine in the reconstituted composition is at least about 5 millimolar (mM). The composition may comprise an excipient or carrier. In some aspects, the composition is a pharmaceutical composition comprising a pharmaceutically acceptable excipient or carrier and a Factor VII or Factor VIIa polypeptide and histidine as a buffering agent, wherein the concentration of histidine in the reconstituted composition is at least about 5 mM.

In another aspect, the invention provides a lyophilized composition suitable for reconstitution with water, comprising a Factor VII or Factor VIIa polypeptide variant (e.g., a FVII or FVIIa polypeptide variant having clotting activity as described herein), and histidine as a buffering agent, wherein the concentration of histidine in the reconstituted composition is at least about 5 mM. The composition may comprise an excipient or carrier. In some aspects, such composition is a pharmaceutical composition comprising a pharmaceutically acceptable excipient or carrier and a Factor VII or Factor VIIa polypeptide variant (e.g., a FVII or FVIIa polypeptide variant having clotting activity as described herein) and histidine as a buffering agent, wherein the concentration of histidine in the reconstituted composition is at least about 5 mM.

In another aspect, the invention provides a method for producing a storage-stable composition comprising a Factor VII or Factor VIIa polypeptide (e.g., rhFVII or rhFVIIa), the method comprising providing a mixture of the Factor VII or Factor VIIa polypeptide with a histidine buffering agent and water to result in an aqueous composition with a histidine concentration of at least 5 mM, and subjecting the resulting aqueous composition to

lyophilization to result in a freeze-dried composition. The composition may comprise an excipient or carrier. In some aspects, such composition is a pharmaceutical composition comprising a pharmaceutically acceptable excipient or carrier.

In another aspect, the invention provides a method for producing a storage-stable composition comprising a Factor VII or Factor VIIa polypeptide variant (e.g., a FVII or FVIIa polypeptide variant having clotting activity as described herein), the method comprising providing a mixture of the Factor VII or Factor VIIa polypeptide variant with a histidine buffering agent and water to result in an aqueous composition with a histidine concentration of at least 5 mM, and subjecting the resulting aqueous composition to lyophilization to result in a freeze-dried composition. The composition may comprise an excipient or carrier. In some aspects, such composition is a pharmaceutical composition comprising a pharmaceutically acceptable excipient or carrier.

In another aspect, the invention provides a method of treating or preventing a condition treatable by administration of a FVII or FVIIa polypeptide or a FVII or FVIIa polypeptide variant, comprising administering to a patient in need thereof a therapeutically effective amount of a composition of the invention comprising a FVII or FVIIa polypeptide (e.g., rhFVII or rhFVIIa) or a FVII or FVIIa polypeptide variant (e.g., a FVII or FVIIa polypeptide variant having clotting activity as described herein), wherein the composition has been reconstituted with water.

In another aspect, the invention provides a method for increasing blood clot formation in a mammal with a disease or condition in which increased blood clot formation is desirable, comprising administering to the mammal a composition of the invention comprising an excipient or carrier and a FVII or FVIIa polypeptide (e.g., rhFVII or rhFVIIa) or a FVII or FVIIa polypeptide variant having activity in an amount effective to increase blood clot formation in the mammal.

Additional aspects of the invention are described below.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the FVIIa activity (U/mg), determined using the "Factor X activation assay" described herein, of a composition of the invention after storage at 40°C for 28 days compared to the same composition in liquid form stored at the same temperature and an

identical lyophilized composition stored at -80°C as a reference. (T0, T7, T14, T21, T28 = analyses performed at 0, 7, 14, 21 and 28 days, respectively).

Figure 2 shows the amount of aggregated protein (in percent dimers and polymers), determined by SEC-HPLC, in a composition of the invention after storage at 40°C for 28 days compared to the same composition in liquid form stored at the same temperature and an identical lyophilized composition stored at -80°C as a reference.

Figure 3 shows the percentage of oxidized protein, determined by RP-HPLC, in a composition of the invention after storage at 40°C for 28 days compared to the same composition in liquid form stored at the same temperature and an identical lyophilized composition stored at -80°C as a reference.

Figure 4 shows the percentage of heavy chain degradation, determined by RP-HPLC, in a composition of the invention after storage at 40°C for 28 days compared to the same composition in liquid form stored at the same temperature and an identical lyophilized composition stored at -80°C as a reference.

Figure 5 shows the relative potency, determined by the PACT assay described herein, in a composition of the invention after storage at different temperatures for up to one year.

Figure 6 shows the amount of aggregated protein (in percent dimers and polymers), determined by SEC-HPLC, in a composition of the invention after storage at different temperatures for up to one year.

Figure 7 shows the degree of heavy chain degradation, determined by RP-HPLC, in a composition of the invention after storage at temperatures of 5°C, 25°C and 40°C for up to one year relative to the same composition stored at -80°C.

Figure 8 shows the osmolality, determined as described below, in a composition of the invention after storage at different temperatures for up to one year.

Figure 9 shows the degree of oxidation, determined as described below, in a composition of the invention after storage at temperatures of 5°C, 25°C and 40°C for up to one year relative to the same composition stored at -80°C.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a stabilized compositions or formulation of a Factor VII or VIIa polypeptide (e.g., rhFVII or rhFVIIa polypeptide) or a Factor FVII or VIIa

polypeptide variant (e.g., a recombinant FVII or FVIIa polypeptide variant having clotting activity as described herein), in particular a storage-stable lyophilized composition or formulation, and, in one aspect, a composition or formulation that is storage-stable at room temperature.

5 The present invention relates to lyophilized compositions or formulations, including pharmaceutical compositions or formulations, suitable for reconstitution with water or another liquid, comprising a Factor VII or Factor VIIa polypeptide (e.g., recombinant hFVII or hFVIIa polypeptide), or a Factor FVII or VIIa polypeptide variant (e.g., a recombinant FVII or FVIIa polypeptide variant having clotting activity as described herein), and a buffering
10 agent selected from histidine, glycine, arginine, citrate, acetate, succinate and phosphate, and salts thereof. In a preferred aspect, the buffering agent is an amino acid, in particular, histidine.

 In one aspect, the invention provides a lyophilized pharmaceutical composition or formulation suitable for reconstitution with water or other media, comprising a Factor VII or
15 Factor VIIa polypeptide (e.g., recombinant hFVII or hFVIIa polypeptide), or a Factor FVII or VIIa polypeptide variant (e.g., recombinant hFVII or hFVIIa polypeptide variant described herein), and histidine as a buffering agent, wherein the concentration of histidine in the reconstituted composition or formulation is at least about 5 mM. As described below in more detail, the composition will typically include one or more additional ingredients, such as a
20 tonicity modifying agent, a divalent cation such as calcium, a surfactant, an antioxidant and/or a preservative.

 Another aspect of the invention relates to a method for producing a storage-stable composition comprising a Factor VII or Factor VIIa polypeptide (e.g., recombinant hFVII or hFVIIa polypeptide) or polypeptide variant thereof, comprising providing a mixture of the
25 Factor VII or Factor VIIa polypeptide or polypeptide variant thereof with a histidine buffering agent and water, and optionally additional ingredients as described below, to result in an aqueous composition with a histidine concentration of at least 5 mM, and subjecting the resulting aqueous composition to lyophilization to result in a freeze-dried composition.

 Further aspects of the invention include to a method of treating or preventing a
30 condition treatable by administration of a Factor VII or VIIa polypeptide (e.g., recombinant hFVII or hFVIIa) or a FVII or FVIIa polypeptide variant (e.g., recombinant FVII or FVIIa polypeptide variant having clotting activity as described further below), the method comprising administering to a patient in need thereof a therapeutically effective amount of a

reconstituted composition of the invention, as well as the use of a composition of the invention for the manufacture of a medicament for treating or preventing a condition treatable by administration of a Factor FVII or FVIIa polypeptide or a FVII or FVIIa polypeptide variant.

5

Definitions

In the description and claims below, the following definitions apply:

The term "FVII" or "FVII polypeptide" generally refers to a FVII molecule provided in single chain form.

10

The term "FVIIa" or "FVIIa polypeptide" refers to a FVIIa polypeptide molecule provided in its activated two-chain form, wherein the peptide bond between R152 and I153 of the single-chain form has been cleaved.

The terms "rFVII" and "rFVIIa" refer to FVII and FVIIa molecules, respectively, produced by recombinant techniques, respectively. These may have the wild-type human
15 sequence or may be variants of the human sequence.

The terms "hFVII" and "hFVIIa" refer to wild-type human FVII and FVIIa, respectively. The polypeptide sequence of the human FVII/FVIIa protein is well-known and is disclosed, e.g., in US 4,784,950 and in Swiss-Prot under accession number P08709; it is also reproduced below as SEQ ID NO:1. The terms "rhFVII" and "rhFVIIa" refer to human
20 FVII and human FVIIa molecules, respectively, produced by recombinant techniques, respectively.

The term "FVII/FVIIa" means FVII or FVIIa.

The term "parent" FVII or FVIIa molecule is intended to indicate the FVII or FVIIa molecule from which a FVII or FVIIa variant is derived by way of, e.g., amino acid
25 substitution, insertion, or deletion. Although a parent FVII or FVIIa polypeptide may be any FVII or FVIIa polypeptide, and thus be derived from any origin, e.g., a non-human mammalian origin, the parent polypeptide is often hFVII or hFVIIa.

A polypeptide "variant" is a polypeptide which differs in one or more amino acid residues from its parent polypeptide, normally in 1-15 amino acid residues (e.g. in 1, 2, 3, 4,
30 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acid residues), such as in 1-12 amino acid residues, 1-10 amino acid residues, e.g., in 1-8, 1-6, 1-5, 1-4, 1-3, or 1-2 amino acid residues, where the difference between the parent polypeptide and the polypeptide variant is, e.g., an amino acid substitution, insertion and/or deletion.

A "polypeptide sequence" is a polymer of amino acids (a protein, polypeptide, etc.) or a character string representing an amino acid polymer, depending on context. Given the degeneracy of the genetic code, one or more nucleic acids, or the complementary nucleic acids thereof, that encode a specific polypeptide sequence can be determined from the
5 polypeptide sequence.

The terminology used for identifying amino acid positions is illustrated as follows: G124 indicates that position 124 is occupied by a glycine residue in the amino acid sequence of human FVII. G124R indicates that the glycine residue of position 124 has been substituted with an arginine residue. Alternative substitutions are indicated with a "/". Multiple
10 substitutions are indicated with a "+", e.g., K143N+N145S/T means an amino acid sequence which comprises a substitution of the lysine residue in position 143 with an asparagine residue and a substitution of the asparagine residue in position 145 with a serine or a threonine residue. Insertion of an additional amino acid residue, e.g., insertion of an alanine residue after G124, is indicated by G124GA.

15 In general, the discussion herein of the compositions of the invention refers to amounts of the various components in a reconstituted composition, i.e. in an aqueous composition prepared by dissolving the lyophilized composition of the invention in a suitable amount of an aqueous liquid, typically sterile water for injection. The amount of water in the compositions after reconstitution will often correspond to the amount of water in the original
20 compositions prior to lyophilization. The amounts of the various components in the compositions of the invention may therefore alternatively be considered to be the amounts of the same components prior to lyophilization, i.e. the compositions defined in the present description and claims may alternatively be characterized as being prepared from or obtainable by lyophilization of an aqueous composition having the indicated component
25 amounts, concentrations or pH values.

Compositions and Methods of the Invention

In one aspect, the present invention provides novel compositions or formulations of at least one FVII or FVIIa polypeptide (e.g., recombinant hFVII or hFVIIa) and/or at least one
30 FVII or FVIIa polypeptide variant described herein (e.g., a FVII or FVIIa polypeptide variant with clotting activity described herein). Some such compositions or formulations are stable during long-term storage (e.g., for a period of at least about 3 months, e.g., at least about 6 months, at least about 1 year, at least about 18 months, at least about 2 years) without

suffering any substantial damage to the FVII or FVIIa polypeptide or FVII or FVIIa polypeptide variant (e.g. proteolysis or other degradation, or aggregation or denaturation, where the degradation, aggregation or denaturation results in a composition that is unsuitable for therapeutic use due to, e.g., reduced efficacy or risk of immunogenicity)

5 According to the present invention, a recombinant Factor VII or VIIa polypeptide (e.g., recombinant hFVII or hFVIIa polypeptide), or a recombinant FVII or FVIIa polypeptide variant, is formulated in a lyophilized composition containing histidine as a buffering agent, preferably in a concentration of at least about 5 mM (where the concentration is based on the reconstituted composition), and typically with one or more additional ingredients such as a
10 tonicity modifying agent, calcium or another divalent cation, a surfactant, an antioxidant or a preservative. Preferably, the composition contains the FVII/FVIIa polypeptide or FVII/FVIIa polypeptide variant in its activated form, e.g., a recombinant human Factor VIIa or a recombinant FVIIa polypeptide variant, respectively.

15 Buffering agent

According to the invention, a composition or formulation of a recombinant FVII or FVIIa polypeptide (e.g., recombinant hFVII or hFVIIa polypeptide) or a recombinant FVII or FVIIa polypeptide variant described herein is provided based on a lyophilized composition that contains histidine as a buffering agent, in particular wherein the reconstituted
20 composition comprises histidine in a concentration of at least about 5 mM, preferably at least about 10 mM, and preferably greater than about 10 mM, for example in a concentration of up to about 100 mM. The concentration of histidine in the reconstituted composition will typically be up to about 50 mM, such as up to about 40 mM, e.g., up to about 30 mM or 25 mM, and will generally be at least about 10 mM, often at least about 12 mM, such as at least
25 about 15 mM. In one embodiment, the histidine buffering agent may be present in the reconstituted composition in a concentration in the range of about 12-50 mM, such as about 12-40 mM, e.g., about 12-30 mM, such as about 15-25 mM. The histidine buffer is preferably prepared by mixing histidine base and a histidine salt, e.g., histidine-HCl, histidine sulfate or histidine-arginine, in order to avoid addition of sodium ions when adjusting the pH.
30 Alternatively, acid or base may be added to the histidine to adjust the pH to the desired value.

pH

The pH of the compositions of the invention is preferably selected so as to minimize pain or discomfort upon administration to patient, i.e. by selecting a fairly neutral pH value, and taking into consideration the influence of pH on, e.g., stability of the FVII or FVIIa polypeptide or polypeptide variant thereof. The pH will generally be in the range of from about 2.5 to about 9.0. Suitable pH ranges may, for example, be from about 2.5 to about 4.0, e.g., about 3.0 or 3.5; from about 4.0 to about 8.0, e.g., from about 5.0 to about 7.0, such as about 5.5, 6.0 or 6.5; or from about 5.0 to about 9.0, e.g., from about 6.0 to about 8.0, such as about 6.0, 6.5, 7.0 or 7.5. In a preferred embodiment, the pH is in the range of from about 5.5 to about 7.0, typically from about 6.0 to about 7.0, such as from about 6.2 to about 6.8, e.g., from about 6.4 to 6.6, such as about 6.5.

Additional ingredients

In addition to the FVII or FVIIa polypeptide and/or FVII or FVIIa polypeptide variant and the buffering agent, the compositions of the invention may include one or more additional excipients or carriers, such as a tonicity modifying agent, a divalent cation such as calcium, a surfactant, an antioxidant and/or a preservative. Pharmaceutical compositions of the invention may include, in addition to the FVII or FVIIa polypeptide and/or FVII or FVIIa polypeptide variant and the buffering agent, one or more additional pharmaceutically acceptable excipients or carriers, such as a tonicity modifying agent, a divalent cation such as calcium, a surfactant, an antioxidant and/or a preservative. Isotonicifiers or tonicity modifying agents are added to ensure isotonicity or to otherwise adjust the tonicity of liquid compositions to a desired level and include polyhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerol, erythritol, arabitol, xylitol, sorbitol or mannitol, sugars such as sucrose or trehalose, or amino acids such as glycine or arginine. Also suitable for use as a tonicity modifying agent are neutral salts such as sodium salts, potassium salts, calcium salts or magnesium salts, e.g., with chloride as the counterion, for example sodium chloride. Mixtures of two or more tonicity modifying agents may also be used. In one embodiment, the tonicity modifying agent is a sugar, preferably sucrose or trehalose, more preferably trehalose. When the tonicity modifying agent is a sugar alcohol or a sugar, the concentration of the tonicity modifying agent in the reconstituted composition will typically be from about 50 mM to about 1000 mM, such as from about 100 mM to about 500 mM, e.g., from about 150 mM to about 300 mM, such as from about 200 mM to about 250 mM. When the tonicity modifying agent is an ionic compound such as a salt or an amino acid, the amount

of the ionic compound will be adapted so as to result in an ionic strength corresponding to the ranges described above for a sugar alcohol or sugar.

In some cases, it may be advantageous to have a high ionic strength in the composition, e.g., at least about 250 mM, at least 300 mM or at least 400 mM, e.g., up to
5 about 500 mM or up to about 1000 mM, for example provided by use of a neutral salt. Such high ionic strength compositions of FVII are described in WO 2004/112828.

Lyoprotectants and/or cryoprotectants may be included in the compositions of the invention in order to protect the protein from stresses that occur during water-removal and freezing, respectively. Cryoprotectants may also serve to protect the protein in its freeze-dried
10 form during storage subsequent to lyophilization. Further, some cryoprotectants may also serve as a lyoprotectant, and some components such as sugar alcohols or sugars that may be included in the composition as a tonicity modifier may in addition function as a lyoprotectant and/or cryoprotectant.

The lyoprotectant may for example be a sugar, for example a disaccharide such as
15 sucrose or trehalose, or an amino acid, such as histidine, arginine and/or glycine. The cryoprotectant may for example be a sugar, in particular a disaccharide such as sucrose or trehalose, or a sugar alcohol such as glycerol, erythritol, arabitol, xylitol, sorbitol or mannitol, or an amino acid, such as histidine, arginine or glycine. In a preferred embodiment, a sugar such as sucrose or trehalose, in particular trehalose, is used as a lyoprotectant/cryoprotectant
20 and as a tonicity modifying agent. In this case, the total amount of the sugar will be as indicated above in the paragraph dealing with tonicity modifying agents.

The composition of the invention will often contain a divalent cation, typically in the form of a calcium salt such as calcium chloride or a magnesium salt such as magnesium chloride, e.g., calcium chloride, to help stabilize the composition. The concentration of the
25 calcium ion or other divalent cation in the reconstituted composition will normally from about 1 mM to about 40 mM, such as from about 2 mM to about 30 mM, e.g., from about 2 mM to about 20 mM, such as from about 5 mM to about 15 mM, e.g., about 10 mM.

Surfactants or detergents, in particular non-ionic surfactants, may be present to help solubilize the polypeptide as well as to protect the polypeptide against surface-induced
30 (agitation or freezing) aggregation, which also reduces the risk of denaturation and aggregation of the protein upon exposure to shear surface stress. Suitable non-ionic surfactants include, for example, polysorbates (polyoxyethylene sorbitan esters, e.g., polysorbate 20 (Tween®-20), polysorbate 80 (Tween®-80), etc.), polyoxamers

(polyoxypropylene-polyoxyethylene block copolymers, e.g., poloxamer 184, poloxamer 188, etc.) and Pluronic® polyols. An example of a preferred non-ionic surfactant is polysorbate 20 (Tween®-20). The surfactant will typically be present in the reconstituted composition in a concentration of from about 0.001% (w/v) to about 0.5%, such as from about 0.001% to about 5 0.05%, e.g., from about 0.005% to about 0.025%, such as about 0.01% or about 0.02%.

In addition, the compositions may comprise miscellaneous excipients such as an antioxidant, for example ascorbic acid, methionine, benzyl alcohol or vitamin E; a bulking agent or filler, e.g., starch; or a chelating agent, e.g., EDTA. In a preferred embodiment the antioxidant is methionine. The concentration of the antioxidant in the reconstituted 10 composition is typically from about 1 mM to about 40 mM, such as from about 2 mM to about 30 mM, e.g., from about 5 mM to about 20 mM, such as about 10 mM.

Preservatives may be added to retard microbial growth, and are typically added in amounts of e.g., about 0.1%-2% (w/v). Suitable preservatives for use with the present invention include phenol, benzyl alcohol, meta-cresol, ortho-cresol, para-cresol, methyl 15 paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalkonium halides (e.g. benzalkonium chloride, bromide or iodide), hexamethonium chloride, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol and 3-pentanol.

In a preferred embodiment, the composition of the invention comprises histidine, trehalose, calcium chloride, Tween®-20 and methionine. More preferably, the composition of 20 this embodiment of the invention in its reconstituted form has a pH of from about 6.0 to about 7.0 and comprises from about 10 mM to about 30 mM histidine, e.g., from about 15 to about 25 mM histidine, from about 150 mM to about 300 mM trehalose, from about 2 mM to about 20 mM calcium chloride, from about 0.001% to about 0.05% (w/v) Tween®-20, and from about 5 mM to about 30 mM methionine.

25 In a further embodiment, the composition does not contain glycylglycine.

Water content

It will be apparent from the above discussion that the compositions of the invention are provided in dry form for long-term storage, since it has been found that the FVII or FVIIa 30 polypeptide (e.g., recombinant hFVII or hFVIIa polypeptide) or a recombinant FVII or FVIIa polypeptide variant described herein is more stable when stored in dry form than when stored in liquid form. It is known in the art that a key determinant in the success of any lyophilization cycle is the amount of residual water found remaining in the lyophilized product. The moisture

content (or “water content”) of the compositions of the invention should therefore be no more than 3% (w/w), preferably no more than 2%, such as no more than about 1%. Methods for determination of the moisture content of lyophilized pharmaceutical compositions are well-known in the art; see for example WO 2003/092731. One example of a well-known technique
5 is Karl Fischer titration, which uses coulometric or volumetric titration to determine trace amounts of water in a sample.

Stability

The compositions of the invention are contemplated to be suitable for long-term
10 storage, e.g., in single- or multi-use vials, pre-filled syringes, ampoules or cartridges that can be stored at a temperature of about 2-8°C, and preferably at a higher temperature such as about 20-25°C, i.e., “room temperature”. The terms “long-term storage” and “storage-stable” refer to the fact that the compositions are able to be stored at a given temperature, for example 2-8°C, such as about 5°C, and more preferably at 20-25°C, for an extended period of time,
15 typically a period of at least about 3 months, preferably at least about 6 months, more preferably at least about 1 year, e.g., at least about 18 months, such as up to about 2 years, without suffering any substantial damage to the polypeptide (e.g. proteolysis or other degradation, or aggregation or denaturation, where the degradation, aggregation or denaturation results in a composition that is unsuitable for therapeutic use due to, e.g.,
20 reduced efficacy or risk of immunogenicity). The storage stability of a protein may, e.g., be measured in terms of activity as compared to a reference composition subjected to the same storage conditions, or compared to the same composition that has not been subject to storage or has been subjected to low-temperature storage at, e.g., about -80°C. The activity measured may, for example, be amidolytic activity or clotting activity, e.g., the activity as determined
25 by the “Factor X activation assay” or “PACT assay” described in the Materials and Methods section below. Suitable assays for determining the amidolytic or clotting activity of a FVII or FVIIa polypeptide or FVII or FVIIa polypeptide variant, as well as other FVII/FVIIa assays, are known in the art and are described, e.g., in WO 01/58935 and WO 03/093465. See also “Whole blood assay” and “Method of measuring the clotting activity” described in the
30 Materials and Methods section below.

The storage stability may alternatively or additionally be measured in terms of the level of aggregate formation by methods known in the art, for example using size-exclusion chromatography, analytical ultracentrifugation or other orthogonal or complementary

methods. The improved storage stability of the compositions of the invention is thus intended to comprise physical stability, e.g., reduced aggregate formation, and/or chemical stability, e.g., reduced degradation or reduced oxidation.

In a preferred embodiment, the storage-stable compositions of the invention are
5 “heat-stable”, meaning that the compositions have stability characteristics as outlined above when stored at an ambient temperature of about 20°C, and preferably when stored at a temperature of about 25°C. The composition of the invention comprising a FVII or FVIIa polypeptide or polypeptide variant thereof will therefore preferably be one which is stable at a temperature of 20°C, and preferably at 25°C, upon storage for a period of at least about 3
10 months, preferably at least about 6 months, more preferably at least about 1 year, e.g., at least about 18 months, such as up to about 2 years.

A composition of the invention may be considered to be “stable” if it fulfills one or more of the following criteria after storage under defined conditions (temperature and time), e.g., as explained above. For the assessment of stability, the stored composition to be tested is
15 compared to a reference that may be either the same composition at time 0 (i.e. at the beginning of the storage period) or the same composition that has been stored in lyophilized form for the same length of time at -80°C. The reference composition will typically be a lyophilized composition stored at -80°C.

- Bioactivity, e.g., determined using the “Factor X activation assay” or “PACT
20 assay” described herein: A bioactivity compared to the reference of at least 80%, preferably at least 85%, more preferably at least 90%, still more preferably at least 95%, e.g., at least 98%.
- Degree of aggregation, e.g., determined using SEC-HPLC as described below: An increase in aggregation compared to the reference of not more
25 than 3% (in absolute terms), preferably not more than 2.5%, more preferably not more than 2%, still more preferably not more than 1.5%, e.g., not more than 1%.
- Oxidation, e.g., as determined by RP-HPLC as described below: Oxidation should not increase more than 5% (in absolute terms) compared to the degree
30 of oxidation of the reference, preferably not more than 3%, more preferably not more than 2%, and most preferably as little as 1% or less.

- Heavy chain degradation, e.g., as determined by RP-HPLC as described below, should not increase more than 5% (in absolute terms) compared to the degree of heavy-chain degradation of the reference, preferably not more than 3%, more preferably not more than 2%, most preferably as little as 1% or less.

The lyophilized composition may, e.g., be packaged in single- or multi-use vials, pre-filled syringes, ampoules or cartridges for reconstitution with a solvent shortly before use. The solvent will typically be sterile water, but may optionally include one or more components that can otherwise be present in the lyophilized composition, e.g., a tonicity modifying agent, a non-ionic surfactant, an antioxidant or a preservative. For ease of administration, a freeze-dried composition according to the invention may alternatively be packaged in a pre-filled, two-compartment syringe wherein one of the compartments contains the freeze-dried composition comprising a FVII or FVIIa polypeptide or a polypeptide variant thereof and the other compartment contains a solvent, typically sterile water, thus allowing the dry composition comprising a FVII or FVIIa polypeptide or a polypeptide variant thereof to be quickly and easily mixed with water in an appropriate amount immediately prior to administration. The water used for reconstitution of the compositions of the invention is sterile water of a type commonly used for this purpose in the pharmaceutical industry, e.g., sterile water for injection (WFI), preserved water, buffered water or saline. The amount of water to be used for reconstitution relative to the amount of FVII or FVIIa polypeptide or polypeptide variant thereof will often, but not necessarily, correspond to the amount of water in the original composition prior to lyophilization. As described below, the concentration of the polypeptide or polypeptide variant in the reconstituted composition of the invention will typically be in the range of about 0.01-10 mg/ml, more typically 0.1-5 mg/ml, such as about 0.2-2 mg/ml, typically about 0.4-1.5 mg/ml, e.g., about 0.5-1.0 mg/ml. If desired, lyophilization can be used as a means for concentrating the FVII or FVIIa polypeptide or polypeptide variant thereof, in which case the amount of water in the reconstituted composition will be less than the amount in the original composition prior to lyophilization.

Polypeptides

The polypeptides that may be formulated according to the present invention include in particular recombinant human FVII or FVIIa polypeptide (the polypeptide sequence of which is shown in SEQ ID NO:1) as well as recombinant FVII or FVIIa polypeptide variants

(e.g., polypeptide variants of the human FVII or FVIIa polypeptide shown in SEQ ID NO:1) thereof, including those described in detail herein, and preferably in the activated form. FVII or FVIIa polypeptide variants of interest include, for example, those described in WO 01/58935, WO 03/093465, WO 2004/029091, WO 2004/111242, WO 99/20767, WO 00/66753, WO 88/10295, WO 92/15686, WO 02/29025, WO 01/70763, WO 01/83725, WO 02/02764, WO 02/22776, WO 02/38162, WO 02/077218, WO 03/027147, WO 03/037932, WO 2004/000366, WO 2004/029090, and WO 2004/108763.

The FVII or FVIIa polypeptide variants may include one or more amino acid substitutions, insertions, and/or deletions compared to the wild-type human FVII or FVIIa polypeptide sequence shown in SEQ ID NO:1, for example, resulting in a polypeptide variant that differs in 1-15 amino acid residues (e.g., in no more than 1-15 amino acid residues) from the amino acid sequence of wild-type human FVII (SEQ ID NO:1), typically in 1-12 or 1-10 amino acid residues, e.g., in 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, or 1-2 amino acid residues or 1 amino acid residue, where the difference(s) in amino acid sequence from the wild-type FVII/FVIIa sequence shown in SEQ ID NO:1 is/are typically one or more amino acid substitutions. Such amino acid substitutions may be performed, e.g., with the aim of introducing one or more glycosylation sites, including N-glycosylation sites (e.g., N-glycosylation sites that may be glycosylated *in vivo* (e.g., via production of the polypeptide in a glycosylating mammalian cell) or by *in vitro* glycosylation (i.e. a synthetic glycosylation performed *in vitro* normally involving covalently linking a carbohydrate molecule to an attachment group of the polypeptide variant, optionally using a cross-linking agent), or introducing one or more PEGylation sites into the polypeptide, and/or for improving or otherwise modifying the clotting activity of the wild-type polypeptide, for example, by way of one or more amino acid substitutions in the Gla domain (amino acid residues 1-45 of hFVIIa) to improve clotting activity. The FVII or FVIIa polypeptide variants typically have clotting activity. Clotting activity can be measured using one of the clotting assays described herein or one of the many clotting assays known in the art. Exemplary FVII or FVIIa polypeptide variants having clotting activity are discussed below and are described in more detail in WO 01/58935, WO 03/093465, WO 2004/029091 and WO 2004/111242. Alternatively, where a FVII or FVIIa polypeptide variant is designed to function as an anticoagulant, the composition of the invention may comprise a FVII or FVIIa polypeptide variant having reduced clotting activity.

Preferred FVII or FVIIa polypeptide variants suitable for use in the compositions of the invention include at least one modification in the Gla domain (residues 1-45 of human FVII or FVIIa) and/or at least one amino acid modification that introduces an attachment site for a non-polypeptide moiety. A non-polypeptide moiety includes, e.g., a sugar moiety or a non-polypeptide polymer, such as a polyethylene glycol (PEG) polymer. Non-limiting
5 examples of such modifications are provided in the following.

Modification of the Gla domain: In one embodiment, the invention provides a recombinant FVII or FVIIa polypeptide variant having clotting activity which comprises a polypeptide sequence that differs from the polypeptide sequence of hFVII or hFVIIa in SEQ
10 ID NO:1 in more than 15 amino acid residues (e.g., no more than 12, 10, 8, 7, 6, 5, 4, 3, 2, 1 amino acid residues), the polypeptide variant having at least one amino acid modification in the Gla domain, in particular at least one modification that results in increased phospholipid membrane binding affinity compared to a similar polypeptide without said modification in the Gla domain. Such modifications in the Gla domain are disclosed, e.g., in WO 99/20767, WO
15 00/66753 and WO 03/093465, and include modifications in one or more of positions 10, 11, 28, 32, 33, and 34 relative to SEQ ID NO:1. The amino acid positions in the variant are typically numbered according to the amino acid positions of SEQ IDNO:1. Preferably, the variant includes modifications in at least position 10 or 32, preferably both. The variant may thus include substitution of a glutamine, a glutamic acid, an aspartic acid or an asparagine
20 residue in position 10, preferably a glutamine residue; and/or substitution of a glutamic acid or an aspartic acid residue in position 32, preferably a glutamic acid. Preferably, the variant includes substitutions at both of positions 10 and 32, more preferably the substitutions P10Q+K32E.

In another embodiment, the invention provides a recombinant FVII or FVIIa
25 polypeptide variant having clotting activity which comprises a polypeptide sequence that differs from the polypeptide sequence of hFVII or hFVIIa in SEQ ID NO:1 in more than 15 amino acid residues (e.g., no more than 12, 10, 8, 7, 6, 5, 4, 3, 2, 1 amino acid residues), the polypeptide variant having one or more amino acid at least one amino acid substitution in amino acid position 10, 11, 28, 32, 33, 34, and/or 36 of SEQ ID NO:1, wherein the amino
30 acid positions in the variant are numbered according to SEQ ID NO:1.

In another embodiment, the FVII or FVIIa polypeptide variant having clotting activity includes an amino acid substitution of a glutamic acid or a phenylalanine residue at position 28 of SEQ ID NO:1; or substitution of a hydrophobic amino acid residue in position

33, the substitution being selected from the group consisting of D33I, D33L, D33M, D33V, D33F, D33Y and D33W, in particular D33F, of SEQ ID NO:1.

In another embodiment, the FVII or FVIIa polypeptide variant having clotting activity includes an amino acid substitution of a negatively charged residue in position 34, i.e. A34E or A34D, preferably A34E, of SEQ ID NO:1. Alternatively, the variant may include a hydrophobic amino acid residue introduced by substitution in position 34 of SEQ ID NO:1. In this case, the hydrophobic amino acid residue to be introduced in position 34 of SEQ ID NO:1 may be selected from the group consisting of I, L, M, V, F, Y and W. Preferred substitutions in this position include A34E and A34L.

In another embodiment, the FVII or FVIIa polypeptide variant having clotting activity includes an amino acid substitution in position 36 of SEQ ID NO:1. Preferably, the amino acid residue to be introduced by substitution in position 36 is a negatively charged amino acid residue, i.e. R36E or R36D, in particular R36E.

In another embodiment, the FVII or FVIIa polypeptide variant having clotting activity includes an amino acid substitution in position 38 of SEQ ID NO:1, in particular, a negatively charged amino acid residue introduced by substitution in position 38, i.e. K38E or K38D, in particular K38E.

In another embodiment, the FVII or FVIIa polypeptide variant includes an insertion of at least one (typically one) amino acid residue between position 3 and 4 of SEQ ID NO:1.

The inserted amino acid residue is preferably a hydrophobic amino acid residue. An exemplary insertion comprises the introduction of a tyrosine residue between the amino acid residue at position 3 (alanine) and the amino acid residue at position 4 (phenylalanine) of SEQ ID NO:1, which insertion is designated as A3AY.

Specific examples of such FVII or FVIIa polypeptide variants having multiple substitutions in the Gla domain include: P10Q+K32E; P10Q+K32E+A34E; P10Q+K32E+R36E; P10Q+K32E+K38E; P10Q+K32E+A34E+R36E; P10Q+K32E+R36E+K38E; P10Q+K32E+A34E+K38E; P10Q+K32E+A34E+R36E+K38E; P10Q+K32E+A34L; P10Q+K32E+A34L+R36E; P10Q+K32E+A34L+K38E; and P10Q+K32E+A34L+R36E+K38E relative to SEQ ID NO:1. The amino acid positions in the variant are numbered according to SEQ ID NO:1. Many such variants have clotting activity. Clotting activity can be measured using one of the clotting assays described herein or one of the many clotting assays known in the art.

Introduction of glycosylation sites: In another embodiment, the FVII or FVIIa polypeptide variants, including those having clotting activity, used in the compositions of the invention comprise a polypeptide sequence comprising one or more amino acid modifications, typically amino acid substitutions, that introduce an N-glycosylation site (including, e.g., an *in vivo* N-glycosylation site) compared to hFVII or hFVIIa with the wild-type sequence (SEQ ID NO:1). An N-glycosylation site has the sequence N-X-S/T/C, wherein X is any amino acid residue except proline, N is asparagine and S/T/C is either serine, threonine or cysteine, preferably serine or threonine, and most preferably threonine. Attachment sites for N-glycosylation, including, e.g., *in vivo* N-glycosylation, can therefore be introduced by amino acid modification, typically amino acid substitution, of one or two amino acid residues in order to obtain the necessary N-X-S/T/C triplet in the variant sequence.

A FVII or FVIIa polypeptide variant having one or more introduced N-glycosylation sites relative to the hFVII or hFVIIa polypeptide sequence (SEQ ID NO:1) may comprise 1-5 additional N-glycosylation sites, such as 1-4 or 1-3 additional N-glycosylation sites, e.g., 1, 2 or 3 additional N-glycosylation sites relative to the native hFVII/hFVIIa sequence (SEQ ID NO:1). In one particular aspect, a FVII or FVIIa polypeptide variants may comprise 1-5 additional *in vivo* N-glycosylation sites, such as 1-4 or 1-3 additional *in vivo* N-glycosylation sites, e.g., 1, 2 or 3 additional *in vivo* N-glycosylation sites relative to the native hFVII/hFVIIa sequence. Human FVII or FVIIa has four naturally-occurring glycosylation sites at positions N145, N322, S52 and S60, where S52 and S60 are O-glycosylation sites and N145 and N322 are N-glycosylation sites. Attachment of sugar moieties to one or more N-glycosylation sites may be performed *in vitro* (e.g., by a known synthetic glycosylation technique performed *in vitro*, which typically involves covalently linking a carbohydrate molecule to an attachment group of the polypeptide variant, optionally using a cross-linking agent) or by expression of the polypeptide variant in a host cell capable of *in vivo* glycosylation (e.g., a eukaryotic cell, such as a yeast, fungal or mammalian cell. Exemplary mammalian cells include COS cells, CHO cells (e.g., CHO-1 cells), BHK cells, HEK cells, and the like.

Specific examples of substitutions creating an N-glycosylation site (e.g., *in vivo* N-glycosylation site) introduced by amino acid substitution(s) include amino acid substitutions selected from the group consisting of A51N, G58N, T106N, K109N, G124N, K143N+N145T, A175T, I205S, I205T, V253N, T267N, T267N+S269T, S314N+K316S,

S314N+K316T, R315N+V317S, R315N+V317T, K316N+G318S, K316N+G318T, G318N and D334N, wherein amino acid positions in the variant are numbered according to the positions in SEQ ID NO:1. Preferred amino acid substitutions include one or more of T106N, I205T and V253N.

5 In one embodiment, only one N-glycosylation site (e.g., *in vivo* N-glycosylation site) has been introduced by amino acid substitution. In another embodiment, two or more N-glycosylation sites (e.g., *in vivo* N-glycosylation sites) have been introduced by amino acid substitution. Preferred amino acid substitutions creating two N-glycosylation sites include amino acid substitutions are selected from the group consisting of T106N+A175T,
 10 T106N+I205T, T106N+V253N, T106N+T267N+S269T, A175T+I205T, A175T+V253N, A175T+T267N+S269T, I205T+V253N, I205T+T267N+S269T and V253N+T267N+S269T, more preferably from the group consisting of T106N+I205T, T106N+V253N and I205T+V253N, wherein amino acid positions in the variant are numbered according to the positions in SEQ ID NO:1.

15 In a further embodiment, three or more N-glycosylation sites (e.g., *in vivo* N-glycosylation sites) have been introduced by substitution. Examples of preferred substitutions creating three N-glycosylation sites include substitutions selected from the group consisting of I205T+ V253N+T267N+S269T and T106N+I205T+V253N, wherein amino acid positions in the variant are numbered according to the positions in SEQ ID NO:1.

20 *Polypeptide variants having a modification in the Gla domain and an introduced N-glycosylation site:* In a further embodiment, the compositions of the invention may comprise a FVII or FVIIa polypeptide variant having clotting activity which comprises a polypeptide sequence having at least one modification in the Gla domain and at least one introduced N-glycosylation site (e.g., *in vivo* N-glycosylation site) as described in the respective sections
 25 above.

Specific examples of “combined” FVII or FVIIa polypeptide variants having clotting activity which comprise multiple substitutions in the Gla domain and at least one introduced N-glycosylation site include: P10Q+K32E+T106N; P10Q+K32E+A34E+T106N; P10Q+K32E+R36E+T106N; P10Q+K32E+A34E+R36E+T106N; P10Q+K32E+I205T;
 30 P10Q+K32E+A34E+I205T; P10Q+K32E+R36E+I205T; P10Q+K32E+A34E+R36E+I205T; P10Q+K32E+V253N; P10Q+K32E+A34E+V253N; P10Q+K32E+R36E+V253N; P10Q+K32E+A34E+R36E+V253N; P10Q+K32E+ T106N+I205T; P10Q+K32E+A34E+T106N+I205T; P10Q+K32E+R36E+T106N+I205T;

- P10Q+K32E+A34E+R36E+T106N+I205T; P10Q+K32E +T106N+V253N;
 P10Q+K32E+A34E+T106N+V253N; P10Q+K32E+R36E+T106N+V253N;
 P10Q+K32E+A34E+R36E+T106N+V253N; P10Q+K32E+I205T+V253N;
 P10Q+K32E+A34E+I205T+V253N; P10Q+K32E+R36E+I205T+V253N;
 5 P10Q+K32E+A34E+R36E+I205T+V253N; P10Q+K32E+T106N+I205T+V253N;
 P10Q+K32E+A34E+T106N+I205T+V253N; P10Q+K32E+R36E+T106N+I205T+V253N;
 P10Q+K32E+A34E+R36E+T106N+I205T+V253N; P10Q+K32E+A34L+T106N;
 P10Q+K32E+A34L+I205T; P10Q+K32E+A34L+V253N;
 P10Q+K32E+A34L+T106N+I205T; P10Q+K32E+A34L+T106N+V253N;
 10 P10Q+K32E+A34L+I205T+V253N; P10Q+K32E+A34L+T106N+I205T+V253N;
 P10Q+K32E+A34L+R36E+T106N; P10Q+K32E+A34L+R36E I205T;
 P10Q+K32E+A34L+R36E+V253N; P10Q+K32E+A34L+R36E+T106N+I205T;
 P10Q+K32E+A34L+R36E+T106N+V253N; P10Q+K32E+A34L+R36E+I205T+V253N;
 and P10Q+K32E+A34L+R36E+T106N+I205T+V253N; wherein amino acid positions in the
 15 variant are numbered according to the positions in SEQ ID NO:1.

Polypeptide variants with modifications in the tissue factor binding site: In another embodiment, the compositions of the invention may comprise a FVII or FVIIa polypeptide variant having clotting activity which comprise a polypeptide sequence having an amino acid
 20 substitution in at least one amino acid position in SEQ ID NO:1 selected from the group consisting of L39, I42, S43, K62, L65, F71, E82 and F275, wherein amino acid positions in the variant are numbered according to the positions in SEQ ID NO:1. These amino acid substitutions in the tissue factor (TF) binding site of the FVII/FVIIa molecule result in an improved clotting activity compared to hFVII/hFVIIa. Preferred substitutions in these amino
 25 acid positions in the TF binding site include the following: L39E, L39Q or L39H; I42R; S43Q; K62E or K62R; L65Q or L65S; F71D, F71E, F71N, F71Q or F71Y; E82Q or E82N; F275H, wherein amino acid positions in the variant are numbered according to the positions in SEQ ID NO:1. The FVII or FVIIa polypeptide variant of this embodiment may, e.g., comprise one, two or three of these amino acid substitutions in SEQ ID NO:1. Preferred
 30 substitutions include one or more of S43Q, K62E, L65Q and F71Y, in particular one or more of S43Q, K62E and L65Q, wherein amino acid positions in the variant are numbered according to the positions in SEQ ID NO:1. Further information about variants of this type having modifications in the TF binding site may be found in WO 2004/029091. It will be

understood that these substitutions in the TF binding site may if desired be combined with one or more of the other types of modifications described elsewhere herein, e.g., the modifications in the Gla domain as described above (e.g., amino acid substitution, insertion or deletion), introduction of at least one N-glycosylation site (e.g., *in vivo* N-glycosylation site),
5 and/or conjugation with a PEG polymer as described below.

Polypeptide variants having an introduced PEGylation site: In a further embodiment, the compositions of the invention may comprise a FVII or FVIIa polypeptide variant having at least one polymer molecule, in particular a polyethylene glycol (PEG) or other polyalkylene oxide, conjugated to an attachment group selected from the group
10 consisting of a lysine residue, a cysteine residue, an aspartic acid residue, a glutamic acid residue, a histidine residue, and a tyrosine residue, preferably a cysteine or a lysine residue.

Methods for conjugating various polypeptides with a polyethylene glycol moiety ("PEGylation") are known in the art. For example, WO 01/58935 describes methods by which PEG moieties may be attached to a FVII or FVIIa polypeptide variant which has been
15 modified relative to hFVII or hFVIIa sequence (SEQ ID NO:1) so as to have at least one introduced and/or removed attachment site for PEGylation, for example, one or more introduced lysine residues, optionally in combination with removal of one or more lysine residues in positions where PEGylation is not desired, or one or more introduced cysteine residues, in this case optionally in combination with removal of one or more cysteine
20 residues. Additional information regarding PEGylation may be found, e.g., in WO 02/02764, which discloses vitamin K-dependent polypeptides such as FVIIa linked to a PEG polymer, for example wild-type human FVII or FVIIa and a variant of FVII or FVIIa having the substitutions P10Q and K32E, in WO 96/11953, which describes methods for preparing N-terminally PEGylated proteins, and in the Nektar Advanced PEGylation Catalog 2005-2006,
25 "Polyethylene Glycol and Derivatives for Advanced PEGylation" (Nektar Therapeutics). It will be understood that the FVII or FVIIa polypeptide variants for use in the compositions of the invention may, in addition to the attachment of one or more PEG polymers, also include one or more of the amino acid modifications otherwise described herein to provide, e.g., an increased phospholipid membrane binding affinity and/or an increased tissue factor
30 independent activity, and/or to provide one or more introduced N-glycosylation sites (e.g., introduced *in vivo* N-glycosylation sites).

Other modifications: In a further embodiment, variants for use in the compositions of the invention may comprise, optionally in addition to one or more of the modifications

described above, at least one further amino acid substitution in a position selected from the group consisting of position 74, 77 and 116, in particular P74S, E77A and/or E116D, wherein amino acid positions in the variant are numbered according to the positions in SEQ ID NO:1.

In a still further embodiment, the FVII or FVIIa polypeptide variant may comprising a polypeptide sequence comprising one or more mutations known to increase the intrinsic activity of the polypeptide, for example, those described in WO 02/22776. For example, the variant may comprise at least one amino acid modification (e.g., substitution) in an amino acid position in SEQ ID NO:1 selected from the group consisting of 157, 158, 296, 298, 305, 334, 336, 337 and 374 wherein amino acid positions in the variant are numbered according to the positions in SEQ ID NO:1. Examples of such substitutions include one or more of V158D, E296D, M298Q, L305V and K337A.

The recombinant FVII/FVIIa polypeptide (e.g., hFVII or hFVIIa) or any recombinant FVII or FVIIa polypeptide variant thereof described herein may be produced by any suitable organism, e.g., in mammalian, yeast or bacterial cells, although eukaryotic cells are preferred, more preferably host cells capable of *in vivo* glycosylation, in particular mammalian cells, such as CHO cells, HEK cells or BHK cells. Methods for production of recombinant FVII/FVIIa polypeptides as well as polypeptide variants thereof using, e.g., mammalian cells are well-known in the art, as are methods for subsequent purification and isolation of the recombinant polypeptides. See, for example, WO 01/58935, WO 03/093465 and WO 2005/002615.

The concentration of the FVII or FVIIa polypeptide or FVII or FVIIa polypeptide variant in the compositions of the invention (after reconstitution) may vary and will be in the range of about 0.001-100 mg/ml, e.g., about 0.01-10 mg/ml, typically about 0.1-5 mg/ml, such as about 0.2-2 mg/ml, e.g., about 0.4-1.5 mg/ml, e.g., about 0.5-1.0 mg/ml. For example, the concentration may be similar to that of NovoSeven® after reconstitution with water, which is about 0.6 mg/ml, or slightly higher such as about 1.0 mg/ml. For recombinant FVII or FVIIa polypeptide variants that have clotting activity, the concentration of the polypeptide may in some cases be slightly lower, e.g., about 0.1-0.5 mg/ml, such as about 0.2-0.4 mg/ml.

Additional aspects

In a further aspect, the invention relates to a method for producing a storage-stable composition comprising a FVII or FVIIa polypeptide or polypeptide variant thereof, comprising providing a mixture of the FVII or FVIIa polypeptide or polypeptide variant

thereof with a histidine buffering agent and water to result in an aqueous composition with a histidine concentration of at least 5 mM, and optionally with other ingredients such as one or more of a tonicity modifying agent, calcium or another divalent cation, a surfactant, an antioxidant or a preservative, and subjecting the resulting aqueous composition to

5 lyophilization to result in a freeze-dried composition, e.g., as described elsewhere herein.

Freeze-dried protein compositions of this type are often referred to as "freeze-dried cakes". It will be understood that the nature and amounts of the buffering agent and any other additional components used in this method for stabilizing a composition of a FVII or FVIIa polypeptide or polypeptide variant thereof will be as described above. The filling of formulated drug

10 substance into vials or another type of container such as a pre-filled, two-compartment syringe and the lyophilization process will be dependent on the amount of protein that is to be freeze-dried as well as the type of container in which lyophilization is to be performed.

Suitable methods for protein or polypeptide composition or formulation and lyophilization are described in the literature and are known to persons skilled in the art.

15 In still further aspects, the invention relates to a method of therapeutically or prophylactically treating or preventing a condition in a mammal (e.g., non-human primate, human, mouse, monkey, etc.) treatable by administration of a FVII or FVIIa polypeptide or a FVII or FVIIa polypeptide variant, comprising administering to the mammal in need thereof a therapeutically effective amount of a reconstituted composition of the invention comprising a

20 FVII or FVIIa polypeptide or a FVII or FVIIa polypeptide variant as described herein, as well as use of a composition of the invention comprising a FVII or FVIIa polypeptide or a FVII or FVIIa polypeptide variant as described herein for the manufacture of a medicament for treating or preventing a condition in a mammal treatable by administration of FVII or FVIIa or a polypeptide variant thereof.

25 Conditions prophylactically or therapeutically treatable in a mammal by administration of FVII or FVIIa or a polypeptide variant thereof (and/or by compositions thereof) include, but are not limited to, e.g., blood factor deficiencies, in particular hemophilia A or B, as well as bleeding associated with trauma (both blunt and penetrative trauma), intracerebral hemorrhage (ICH), traumatic brain injury (TBI), burns, variceal bleeds,

30 gastrointestinal bleeding, surgical bleeds, transplantation (e.g., tissue graft or organ transplantation), fibrinolytic treatment, anticoagulant treatment, postpartum hemorrhage, viral-induced hemorrhage, Von Willebrand disease, and thrombocytopenia. See WO

2006/114105 for further information on conditions that may be treated by administration of FVII or FVIIa, or a FVII or FVIIa polypeptide variant.

The invention also provides methods for increasing blood clot formation in a mammal with a disease or condition in which increased blood clot formation is desirable, comprising administering to a mammal in need of increased blood clot formation a composition of the invention comprising at least one FVII or FVIIa polypeptide, or at least one FVII or FVIIa polypeptide variant, in an amount effective to increase blood clot formation in the mammal. Diseases or conditions in which increased blood clot formation is desirable include, but are not limited to, e.g., hemophilia, such as hemophilia A or B, as well as bleeding associated with trauma (both blunt and penetrative trauma), intracerebral hemorrhage (ICH), traumatic brain injury (TBI), burns, variceal bleeds, gastrointestinal bleeding, surgical bleeds, transplantation (e.g., tissue graft or organ transplantation), fibrinolytic treatment, anticoagulant treatment, postpartum hemorrhage, viral-induced hemorrhage, Von Willebrand disease, and thrombocytopenia. See WO 2006/114105 for further information on diseases and conditions in which increased blood clot formation may be facilitated by administration of FVII or FVIIa, or a FVII or FVIIa polypeptide variant.

The compositions of the invention may be administered to a mammal as single or multiple injections (bolus or transfusion), and may, e.g., be administered parenterally, e.g., subcutaneously, intramuscularly or intravenously. Typically, a composition of the invention is administered intravenously to a mammal. The dosage of the polypeptide will, e.g., depend on the condition being treated and the weight of the patient, but will often be similar to dosages used for rhFVIIa (NovoSeven®), for example, from about 20 to about 300 µg/kg, typically from about 25 to about 150 µg/kg, such as from about 40 to about 120 µg/kg

25 **EXAMPLES**

The invention is further described by the following non-limiting examples, which illustrate compositions according to the invention.

Materials and methods

30

Production of recombinant FVIIa polypeptide variant

The recombinant Factor VIIa polypeptide variant used in the examples below was a variant of recombinant hFVIIa. This polypeptide variant comprised the substitutions

P10Q+K32E+A34E+R36E+T106N+V253N compared to the polypeptide sequence of wild-type human FVIIa shown in SEQ ID NO:1. This FVIIa polypeptide variant, which has clotting activity, was produced in CHO-K1 cells substantially as described in WO 2004/111242 and purified substantially as described in WO 2006/074664.

5

Preparation of FVII compositions or formulations

Frozen drug substance produced as outlined above was thawed, sterile filtered, and lyophilized using the lyophilization parameters indicated below in Examples 1 and 3. No adjustment for protein (polypeptide) or excipient concentration was performed.

10

Absorbance

Protein (polypeptide) concentration and percent protein recovery were determined by measuring absorbance at A_{280} . The compositions or formulations were microfuged before absorbance was measured. Analysis was performed in duplicate (150 μ l/well) using a 96-well quartz plate. The respective buffers for each composition or formulation were used to blank the readings. The corrected mg/ml calculations were based on an extinction coefficient of 1.2.

15

pH measurements

The pH readings were done post calibration using a Corning pH Meter 440.

20

Reconstitution time

The lyophilized compositions or formulations were reconstituted in sterile water as follows: the vial was tilted at a 45° angle and the water was dripped down the glass. A timer was started and the vial was rotated and visually inspected for complete dissolution. The timer was stopped when reconstitution was observed by visual inspection.

25

Osmolality

A Vapro® Osmometer calibrated using three standards (100, 290 and 1000 mmol/kg) and tested with a clean test was used for determining osmolality. The following criteria were used to measure the compositions or formulations: 100 ± 2 , 290 ± 3 , 1000 ± 5 and the clean test level below 10. For testing, 10 μ l of the sample was placed on a solute-free paper disk. A fine-wire thermocouple hygrometer was used to measure the difference between the ambient chamber temperature and the dew point temperature within the enclosed space.

30

The difference between these temperatures is the dew point temperature depression, which is a function of solution vapor pressure. During sample readings, a 290 standard was measured for every three samples to ensure that the instrument had maintained calibration.

5 *Degree of aggregation*

The degree of aggregation was determined using SEC-HPLC using a Superdex™ 200 HR 10/300 column from Amersham Bioscience (Cat. #17-1571-01) in 50mM boric acid, 500 mM NaCl pH 8.5. The percent aggregation is calculated as the relative peak area of high molecular forms compared to the total protein peak area. The percent total recovery (%TRC) is calculated by dividing the sum of the peak areas for t_0 by the sum of the peak areas for subsequent time points.

Heavy-chain degradation and oxidation

Heavy-chain degradation and oxidation were determined by analysis of the results of reverse phase HPLC (RP-HPLC) using a Phenomenex Jupiter™ C5, 2.0 x 250 mm, 300 A, 5 micron column. FVIIa polypeptide variant samples were treated with a reducing agent (dithiothreitol) and heated to 75°C for 10 minutes in order to cleave disulphide bonds. It was consequently possible to use RP-HPLC separation in a linear gradient of water and acetonitrile, both acidified with trifluoro acetic acid, to isolate and quantify the relative presence of the following components:

- The light chain of the FVIIa polypeptide variant
- The heavy chain of the FVIIa polypeptide variant
- Four heavy chain degradation products of the FVIIa polypeptide variant
- Three oxidized forms of the heavy chain of the FVIIa polypeptide variant
- The single-chain FVII polypeptide variant, and
- The des-Gla FVIIa polypeptide variant.

Bioactivity (Factor X activation assay)

Bioactivity (potency) for Examples 2 and 3 was determined using a phospholipid-dependent (tissue factor-independent) Factor X activation assay. This assay has been described in detail on page 39826 in Nelsestuen et al., *J Biol Chem*, 2001; 276:39825-39831. Briefly, the FVIIa polypeptide or FVIIa polypeptide variant to be assayed is mixed with a source of phospholipid (preferably a combination of phosphatidylcholine and phosphatidylserine) and

relipidated Factor X in Tris buffer containing BSA. After a specified incubation time the reaction is stopped by addition of excess EDTA. The concentration of Factor Xa is then measured from absorbance change at 405 nm after addition of a chromogenic substrate (S-2765, Chromogenix). After correction for background the tissue factor-independent activity of the FVIIa polypeptide or FVIIa polypeptide variant is determined as the absorbance change after 10 minutes. The specific activity of a FVIIa polypeptide or FVIIa polypeptide variant in U/mg may be found by dividing the result of the Factor X activation assay, expressed in U/ml, by the result of the A_{280} absorbance measurement, expressed in mg/ml, on the same sample.

10 *Bioactivity (PACT assay)*

A Phospholipid-Activated Clotting Time (PACT) assay was used for determining the activity of the samples in Example 4. The PACT assay is a modified "Activated Partial Thromboplastin Time" (APTT) assay, but whereas a conventional APTT assay monitors the contact pathway (also known as the intrinsic pathway) of coagulation, in the PACT assay the contact pathway is inhibited, in part by replacing the kaolin or other silicates used in the APTT assay with phospholipid, thereby preventing conversion of Factor X to Factor Xa via the contact pathway. As a result, thrombin formation and thus the clotting time in the PACT assay is dependent on the interaction between FVIIa polypeptide or FVIIa polypeptide variant and phospholipid.

20 The PACT assay measures tissue factor-independent FVIIa-induced or FVIIa variant-induced clotting of re-calcified citrated human FVII-depleted plasma using phospholipid vesicles as an artificial coagulation activator. Briefly, a fixed amount of homogenized phospholipid vesicles (phosphatidyl serine:phosphatidyl choline:phosphatidyl ethanolamine (20:40:40 ratio); 320 microliters (μ l) of 5 mM sonicated PS:PC:PE phospholipid vesicles per 8 milliliters (ml) of plasma) is added to citrated human FVII-depleted plasma. Subsequently, the sample is added (for example using a FVIIa polypeptide concentration, or a FVIIa polypeptide variant concentration, of between 0.625 nM and 10 nM) and the clotting reaction is started by addition of calcium ions (50 μ l of calcium buffer (containing 19.2 mM HEPES, 144 mM NaCl, pH 7.35, 40mM CaCl_2 , 57.6 milligrams/millilitres [mg/ml] BSA) to 100 μ l of plasma). Clotting is monitored spectrophotometrically at 325 nanometers (nm) in real time. The clotting time is the elapsed time from the start of the reaction by addition of calcium ions to clot detection as determined by the absorbance at 325 nm. A reference standard is ascribed a potency of 1.00, and the

potency of a sample is determined relative to this standard by measuring the relative amount of sample in comparison to the amount of standard that is required to obtain the same PACT value.

5 *Whole blood assay*

The clotting activity of FVIIa and FVIIa polypeptide variants thereof were measured in one-stage assays and the clotting times were recorded on a Thrombotrack IV coagulometer (Medinor). 100 µl of FVIIa or a FVIIa polypeptide variant were diluted in a buffer containing 10 mM glycylglycine, 50 mM NaCl, 37.5 mM CaCl₂, pH 7.35 and transferred to the reaction
10 cup. The clotting reaction was initiated by addition of 50 µl blood containing 10% 0.13 M tri-sodium citrate as anticoagulant. Data were analysed using Excel or PRISM software. The clotting activity reflects the time needed to obtain clot formation. Thus, a lower clotting time corresponds to a higher clotting activity.

15 *Method of measuring the clotting activity*

Clotting activity of FVIIa or a FVIIa polypeptide variant is measured using a standard one-stage clotting assay essentially as described in WO92/15686. Briefly, the sample to be tested is diluted in 50 mM Tris (pH 7.5), 0.1% BSA and 100 µl is incubated with 100 µl of FVII deficient plasma and 200 µl of thromboplastin C containing 10 mM Ca⁺⁺. Clotting
20 times are measured and compared to a standard curve using a pool of citrated normal human plasma in serial dilution.

Method of measuring the anticoagulant activity

The anticoagulant activity of an inactive FVII/FVIIa polypeptide or variant thereof
25 can be measured using the one-stage clotting assay described above (*Method of measuring the clotting activity*) where the inactive conjugate competes with wild-type FVII for a limited amount of relipidated tissue factor. The assay is performed essentially as described in WO 92/15686, Example III, which is hereby incorporated as reference. The ability of the inactive conjugate to prolong the clotting time of wild-type FVII is recorded and taken as a measure of
30 anticoagulant activity.

Moisture content

Lyophilized cakes were analyzed for moisture content by Karl Fischer analysis using a Mettler Toledo DL36 KF Coulometer with a Mettler Toledo DO305 Drying Oven.

Example 1

- 5 In this example, the suitability of histidine, succinate and acetate as the buffering agent was compared. The term "No." in the table below refers to the identifying number given to the composition or formulation. The compositions or formulations tested were as follows:

No.	Sodium succinate	Sodium acetate	Histidine	Trehalose	Tween® 20 (w/v)	CaCl ₂	pH
1	5 mM			250 mM	0.01%	10 mM	5.5
2	5 mM			250 mM	0.01%	10 mM	6.0
3			20 mM	241 mM	0.01%	10 mM	5.5
4			20 mM	241 mM	0.01%	10 mM	6.0
5			20 mM	241 mM	0.01%	10 mM	6.5
6		20 mM		220 mM	0.01%	10 mM	5.5
7		20 mM		220 mM	0.01%	10 mM	6.0

- 10 After dialysis of the aqueous compositions or formulations, 1 ml of each composition or formulation, containing 0.5 mg/ml of the FVII or FVIIa polypeptide variant, was filled into 3 ml lyophilization vials (20 vials for each formulation) and lyophilized according to the following parameters:

Step	Temperature	Pressure	Duration	Ramp Rate
Precooling	+5°C	Atmospheric	Until loading	-
Freezing	-40 °C	Atmospheric	3 hours	1.0 °C / min
Primary Drying	-25 °C	75 milliTor (mTorr)	35 hours*	0.5°C / min
Secondary Drying	25 °C	100 mTorr	6 hours	0.2 °C / min

- 15 * Until the product temperature reached the shelf temperature

After secondary drying, all vials were backfilled with nitrogen at 600 mTorr and stoppered (13 mm West stoppers). The lyophilized compositions or formulations were stored at 40°C and subjected to various analyses, including absorbance (A₂₈₀, for determination of % protein recovery), pH, reconstitution time and osmolality, at 0, 7, 14 and 28 days. In addition, the degree of aggregation was determined using SEC-HPLC; heavy-chain degradation and oxidation were determined by RP-HPLC; and potency was determined with a Factor X activation assay.

Results

With the exception of the two succinate compositions or formulations, all of the compositions or formulations showed acceptable protein recovery (A_{280}) and osmolality after
5 up to 28 days at 40°C. When tested after 14 days, however, the succinate compositions or formulations had a very low A_{280} and osmolality compared to the other compositions or formulations. This is believed to be due to precipitation of calcium succinate.

The compositions or formulations buffered with succinate or histidine showed acceptable pH stability for up to 28 days, while the pH of the compositions or formulations
10 buffered with acetate was unstable, showing relatively large pH increases from the beginning.

All of the lyophilized compositions or formulations could be easily reconstituted within 30 seconds when stored for up to 28 days at 40°C.

In view of the above results it was concluded that histidine is advantageous over either sodium acetate or sodium succinate as a buffering agent. Although the degree of
15 aggregation of the histidine compositions or formulations was acceptable at all three pH values 5.5, 6.0 and 6.5, the SEC-HPLC results for the histidine compositions or formulations showed that there was a slightly decreased aggregation as pH increased. The histidine compositions or formulation was found to be stable at pH 6.5 as assessed by SEC-HPLC (aggregation), RP-HPLC (heavy-chain degradation and oxidation), and the Factor X
20 activation assay (potency). Histidine at pH 6.5 was therefore chosen as the buffering agent for further experiments.

For the compositions or formulations containing either sodium acetate or histidine as a buffer, compositions or formulations identical to those above but containing sucrose instead of trehalose as a tonicity modifying agent (with the amount of sucrose being the same as the
25 amount of trehalose indicated in the table above for the respective compositions or formulations) were also tested. The results for the compositions or formulations containing sucrose were comparable to those containing trehalose. Trehalose was chosen as the tonicity modifying agent in the subsequent experiments, however, in order to reduce any potential increased risk of formation of oxidation products in the presence of sucrose.

30

Example 2

The following aqueous compositions or formulations were prepared and subjected to lyophilization as described above in Example 1. Each of the compositions or formulations

contained 0.6 mg/ml of the FVII or FVIIa polypeptide variant and had a pH of 6.5. The term "No." in the table below refers to the identifying number given to the composition or formulation.

No.	Histidine	Trehalose	Methionine	Tween® 20 (w/v)	CaCl ₂
1	20 mM	264 mM	-	0.005%	2 mM
2	20 mM	254 mM	10 mM	0.005%	2 mM
3	20 mM	240 mM	-	0.005%	10 mM
4	20 mM	230 mM	10 mM	0.005%	10 mM
5	20 mM	264 mM	-	0.01%	2 mM
6	20 mM	254 mM	10 mM	0.01%	2 mM
7	20 mM	240 mM	-	0.01%	10 mM
8	20 mM	230 mM	10 mM	0.01%	10 mM
9	20 mM	264 mM	-	0.02%	2 mM
10	20 mM	254mM	10 mM	0.02%	2 mM
11	20 mM	240mM	-	0.02%	10 mM
12	20 mM	230mM	10 mM	0.02%	10 mM

5

The vials were stored at 40°C for 28 days and analyzed at 0, 14 and 28 days as described above in Example 1. For comparison purposes, the same compositions or formulations in liquid form were stored at 40°C for 21 days and analyzed at 0, 7 and 21 days. The specific activity (U/mg) was also determined at 0, 14 and 28 days for the lyophilized compositions or formulations and at 0, 7 and 21 days for the liquid compositions or formulations. In addition, two vials of each liquid compositions or formulation were frozen at -80°C for freeze-thaw analysis.

Results

15

The appearance of the freeze-dried cake in the lyophilized vials was uniform among the compositions or formulations with the exception of composition or formulation No. 10. All of the lyophilized compositions or formulations could be easily reconstituted within about 30 seconds or less after being stored for 28 days at 40°C.

The lyophilized compositions or formulations after incubation for 28 days at 40°C had a lower degree of aggregation and a higher recovery than the liquid compositions or formulations stored at the same temperature for 21 days. The freeze-thaw stress treatment of the liquid compositions or formulations did not appear to affect the protein. It was found that the liquid compositions or formulations containing 10mM CaCl₂ had a lower degree of

aggregates after 7 days and 21 days than comparable compositions or formulations containing only 2 mM CaCl_2 . As expected, the overall data as analyzed by SE-HPLC show that the lyophilized compositions or formulations are more stable than the liquid compositions or formulations.

5 The lyophilized compositions or formulations had less than a 1% increase in oxidation at 14 and 28 days compared to day 0, with the compositions or formulations containing methionine showing a slightly lower increase in oxidation (0.2-0.5%) compared to the compositions or formulations without methionine.

10 No effect of different amounts of Tween®-20 was seen in either the liquid or the lyophilized compositions or formulations. However, a slightly higher amount of Tween®-20 (e.g. 0.01% rather than 0.005%) or other surfactant might be advantageous in order to ensure that the surfactant is present in the composition or formulation after filtration or other purification steps prior to lyophilization.

15 The moisture content of the lyophilized compositions or formulations was measured at 0 and 28 days. With the exception of one composition or formulation (No. 7) at 28 days, the moisture content at both 0 and 28 days was less than 3% and in many cases less than 2%. The difference in moisture content for each composition or formulation between 0 and 28 days was, with the exception of formulation No. 7, less than 1%.

20 For the liquid compositions or formulations stored at 40°C, the specific activity decreased rapidly, falling on average to just over half of the initial value (54%) after 7 days and to about 18% after 21 days. In contrast, the specific activity of the lyophilized compositions or formulations stored at 40°C for 14 or 28 days remained close to that of the activity determined at day 0, on average 82% when measured at day 14 and 90% at day 28. The most promising composition or formulation (No. 9) had 98.7% of the initial specific
25 activity after 28 days at 40°C. The specific activity for the liquid compositions or formulations containing 10 mM CaCl_2 was higher than for the liquid compositions or formulations containing only 2 mM CaCl_2 , but this was not the case for the lyophilized compositions or formulations.

30

Example 3

A basic composition or formulation comprising the FVII or FVIIa polypeptide variant (366 mg polypeptide variant at a concentration of 0.62 mg variant/ml solvent) was

prepared in 20 mM histidine, pH 6.5, 230 mM trehalose, 10mM CaCl₂, 10 mM methionine and 0.005% Tween-20®. A composition or formulation corresponding to composition or formulation No. 8 of Example 2 was prepared by adding 2.783 ml 1% weight/volume (w/v) of a stock solution of Tween-20® to 185.5 ml of the basic FVII/FVIIa variant composition or formulation and filtering through a 0.2 micrometer (µm) membrane, enough for thirty-five 20 ml lyophilization vials (Mueller & Mueller). The remaining basic composition or formulation, 249.5 ml, was formulated to correspond to composition or formulation No. 10 of Example 2 by adding 1248 µl of the 1% (w/v) Tween-20® stock solution. This was enough for forty-nine analogous 20 ml lyophilization vials. Lyophilization was performed according to the following parameters:

Step	Temperature	Pressure	Duration	Ramp Rate
Precooling	+5°C	Atmospheric	1 hour	-
Freezing	-40 °C	Atmospheric	4 hours	1.0 °C / minute
Primary Drying	-25 °C	60 milliTorr (mTorr)	60 h, 42 minutes*	0.5°C / minute
Secondary Drying	25 °C	60 mTorr	6 hours	0.2 °C / minute

* When the temperature probe in a 5.3 ml placebo vial (without FVII or FVIIa variant) reached -25°C primary drying was extended 10 hours and then ramping to 25°C began.

After secondary drying, all vials were backfilled with nitrogen at 600 mTorr and stoppered (20 mm Daikyo stoppers). These compositions or formulations were stored at either 40°C or -80°C (-80°C = reference composition or reference formulation) and then reconstituted with 5.0 ml of water and analyzed after 14 and 28 days as described above in Examples 1 and 2. In addition, identical compositions or formulations stored in liquid form at 40°C were analyzed for comparison purposes at 0, 7 and 21 days. The lyophilized compositions or formulations of the invention stored at 40°C for 28 days were found to perform well in all tested parameters, including:

- Cake appearance
- Moisture content: less than 2% at day 0 and after 28 days
- Reconstitution time: 13-20 seconds (sec)
- pH stability
- Oxidation
- Bioactivity: Both lyophilized compositions or formulations maintained their full initial specific activity after 14 or 28 days at 40°C.

- Aggregate level: The aggregate levels for both compositions or formulations stored for 28 days at 40°C were very similar to the initial levels and to the levels of the same compositions or formulations stored at -80°C for 40 days, indicating good stability.

The results of the analyses of the lyophilized composition or formulation of the invention stored at 40°C for specific activity, aggregate formation, oxidation and heavy chain degradation are shown in Figures 1-4 for the above composition or formulation of the invention containing 0.01% Tween®-20 compared to the results obtained for the liquid composition or formulation stored at 40°C as well as the same lyophilized composition or formulation stored at -80°C. These figures show that the lyophilized composition or formulation of the invention is fully stable after 28 days of storage at 40°C, exhibiting levels of specific activity, aggregate formation, oxidation and heavy chain degradation that are just as good as for the reference composition or formulation stored at -80°C. In contrast, the liquid composition or formulation exhibits a substantial loss in activity and increase in aggregate formation, oxidation and heavy chain degradation after 21 days at 40°C.

Example 4

A long-term stability study was performed at four different temperatures using the same FVII or FVIIa polypeptide variant used in the other examples and a composition or formulation corresponding to that of Example 3 and composition or formulation No. 8 of Example 2 (20 mM histidine, 10 mM CaCl₂, 230 mM trehalose, 10 mM methionine, 0.01 % Tween®20, pH 6.5). Stability studies were performed on the lyophilized drug product stored at nominal temperatures of -80°C, 5°C, 25°C and 40°C. All filled vials were stored in an upright position and protected from light during the study. Samples were reconstituted and analyzed at the following time points: 15 days, 37 days, 88 days, 191 days, 268 days and 365 days. Samples stored at 25°C and 40°C were cooled to 5°C for transport approximately two weeks prior to reconstitution and analysis. For these samples the time at which the samples were cooled to 5°C is used (28 days, 66 days and 175 days, respectively, instead of 37, 88 and 191 days). Only the samples stored at 5°C and -80°C were analyzed after the first half year.

Data for the relative potency (PACT assay), amount of aggregated protein (percent dimers and polymers), heavy chain degradation (relative to the -80°C samples), osmolality, and oxidation (relative to the -80°C samples), determined as described above, is shown in

Figures 5, 6, 7, 8 and 9, respectively. In each of these figures, the dotted lines indicate two standard deviations above and below the average value for each figure, while the horizontal line between the dotted lines represents the average for all of the measurements. For the determination of relative potency (Figure 5), each data point represents the average of three samples. For Figures 6-9, each data point is the result of assaying a single sample.

Overall, the data provided in Figures 5-9 shows that the FVIIa variant, when stored in the composition of the invention, is stable at the tested temperatures for the entire duration of the study, i.e. for up to one year. This applies to the activity of the variant as shown in Figure 5 as well as to the chemical and physical stability as shown in Figures 6, 7, 8 and 9. It is interesting to note that even at relatively high temperatures of 25°C and 40°C, the FVIIa variant appeared to be highly stable in this composition, which suggests that the composition of the invention is suitable for long-term storage of FVIIa at ambient temperature. For the samples that were stored for up to one year, no significant differences were observed between the composition stored at 5°C and that stored at -80°C. The results of this example thus demonstrate that the composition of the invention provides long-term storage stability for FVIIa under a wide range of temperature conditions.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, patent applications, and/or other documents cited in this application are incorporated herein by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, and/or other document were individually indicated to be incorporated herein by reference in its entirety for all purposes.

WHAT IS CLAIMED IS:

1. A lyophilized pharmaceutical composition suitable for reconstitution with water, comprising a Factor VII or Factor VIIa polypeptide and histidine as a buffering agent,
5 wherein the concentration of histidine in the reconstituted composition is at least about 5 mM.
2. The composition of claim 1, wherein the concentration of histidine in the reconstituted composition is at least about 10 mM.
- 10 3. The composition of claim 1, wherein the concentration of histidine in the reconstituted composition is from about 12 mM to about 50 mM, e.g. from about 12 mM to about 40 mM, such as from about 15 mM to about 25 mM.
4. The composition of any of the preceding claims, further comprising a tonicity
15 modifying agent.
5. The composition of claim 4, wherein the tonicity modifying agent is selected from the group consisting of polyhydric sugar alcohols such as glycerol, erythritol, arabitol, xylitol, sorbitol or mannitol; sugars such as sucrose or trehalose; amino acids such as glycine; and
20 neutral salts such as sodium salts, potassium salts, calcium salts or magnesium salts.
6. The composition of claim 5, wherein the tonicity modifying agent is trehalose.
7. The composition of any of claims 4-6, wherein the concentration of tonicity
25 modifying agent in the reconstituted composition is from about 50 mM to about 1000 mM, such as from about 100 mM to about 500 mM, e.g. from about 150 mM to about 300 mM.
8. The composition of any of the preceding claims, further comprising a calcium or magnesium salt.
30
9. The composition of claim 8, comprising calcium chloride.

10. The composition of claim 8 or 9, wherein the concentration of the calcium or magnesium salt in the reconstituted composition is from about 1 mM to about 40 mM, such as from about 2 mM to about 30 mM, e.g. from about 5 mM to about 20 mM.

5 11. The composition of any of the preceding claims, further comprising an antioxidant.

12. The composition of claim 11, wherein the antioxidant is selected from the group consisting of ascorbic acid, methionine, benzyl alcohol and vitamin E.

10 13. The composition of claim 12, wherein the antioxidant is methionine.

14. The composition of claim any of claims 11-13, wherein the concentration of the antioxidant in the reconstituted composition is from about 1 mM to about 40 mM, such as from about 2 mM to about 30 mM, e.g. from about 5 mM to about 20 mM.

15

15. The composition of any of the preceding claims, further comprising a surfactant.

16. The composition of claim 15, wherein the surfactant is a non-ionic surfactant.

20 17. The composition of claim 16, wherein the non-ionic surfactant is selected from the group consisting of polysorbates, polyoxamers and Pluronic® polyols.

18. The composition of claim 17, wherein the non-ionic surfactant is Tween®-20, Tween®-80 or another polysorbate.

25

19. The composition of any of claims 16-18, wherein the concentration of the non-ionic surfactant in the reconstituted composition is from about 0.001% (w/v) to about 0.5%, such as from about 0.001% to about 0.05%.

30 20. The composition of any of the preceding claims, wherein the pH of the reconstituted composition is from about 5.0 to about 7.0.

21. The composition of claim 20, wherein the pH of the reconstituted composition is about 5.5, about 6.0 or about 6.5.

22. The composition of claim 20, wherein the pH of the reconstituted composition is
5 from about 6.0 to about 7.0, such as from about 6.2 to about 6.8, e.g. from about 6.4 to 6.6.

23. The composition of any of the preceding claims, wherein the concentration of the FVII or FVIIa polypeptide in the reconstituted composition is about 0.1-5 mg/ml, such as about 0.2-2 mg/ml, typically about 0.4-1.5 mg/ml, e.g. about 0.5-1.0 mg/ml.

10

24. The composition of any of the preceding claims, wherein the polypeptide is recombinant human FVIIa or a variant thereof.

25. The composition of claim 1, wherein the composition in reconstituted form has a pH
15 of from about 6.0 to about 7.0 and comprises from about 10 mM to about 30 mM histidine, from about 150 mM to about 300 mM trehalose, from about 2 mM to about 20 mM calcium chloride, from about 0.001% to about 0.05% (w/v) Tween®-20, and from about 5 mM to about 30 mM methionine.

20 26. The composition of any of the preceding claims, wherein the composition does not contain glycylglycine.

27. The composition of any of the preceding claims, wherein the moisture content is no more than 3% (w/w).

25

28. The composition of any of the preceding claims, which exhibits one or more of the following characteristics when analyzed after storage for 6 months at 2-8°C compared to an identical lyophilized reference composition stored for the same period of time at -80°C:

- a) a bioactivity determined using the "Factor X activation assay" described herein that
30 is at least 80% of the bioactivity of the reference composition,
- b) a degree of aggregation as determined using SEC-HPLC that is no more than 3% higher than the degree of aggregation of the reference composition,

- c) a degree of oxidation determined using RP-HPLC that is no more than 5% greater than the degree of oxidation of the reference composition, and
- d) a degree of heavy-chain degradation determined using RP-HPLC that is no more than 5% greater than the degree of heavy-chain degradation of the reference composition.

29. The composition of claim 28, which exhibits one or more of said characteristics when analyzed after storage for 1 year at 2-8°C compared to an identical lyophilized reference composition stored for the same period of time at -80°C.

30. The composition of claim 28, which exhibits all of said characteristics when analyzed after storage for 1 year at 2-8°C compared to an identical lyophilized reference composition stored for the same period of time at -80°C.

31. The composition of claim 28, which exhibits one or more of said characteristics when analyzed after storage for 6 months at 20°C compared to an identical lyophilized reference composition stored for the same period of time at -80°C.

32. The composition of claim 28, which exhibits all of said characteristics when analyzed after storage for 6 months at 20°C compared to an identical lyophilized reference composition stored for the same period of time at -80°C.

33. A method for producing a storage-stable composition comprising a Factor VII or Factor VIIa polypeptide, comprising providing a mixture of the Factor VII or Factor VIIa polypeptide with a histidine buffering agent and water to result in an aqueous composition with a histidine concentration of at least 5 mM, and subjecting the resulting aqueous composition to lyophilization to result in a freeze-dried composition.

34. The method of claim 33, wherein the aqueous composition further comprises one or more ingredients selected from a tonicity modifying agent, a calcium or magnesium salt, a non-ionic surfactant, an antioxidant and a preservative prior to lyophilization.

35. A method of treating or preventing a condition treatable by administration of Factor VIIa, comprising administering to a patient in need thereof a therapeutically effective amount of a composition according to any of claims 1-32, wherein the composition has been reconstituted with water.

5

36. Use of a composition according to any of claims 1-32 for the manufacture of a medicament for treating or preventing a condition treatable by administration of Factor VIIa.

10 37. A method for increasing blood clot formation in a mammal with a disease or condition in which increased blood clot formation is desirable, comprising administering to the mammal a composition of claim 1 in an amount effective to increase blood clot formation in the mammal.

15 38. The composition of claim 1, wherein the FVII or FVIIa polypeptide or FVII or FVIIa polypeptide variant has clotting activity.

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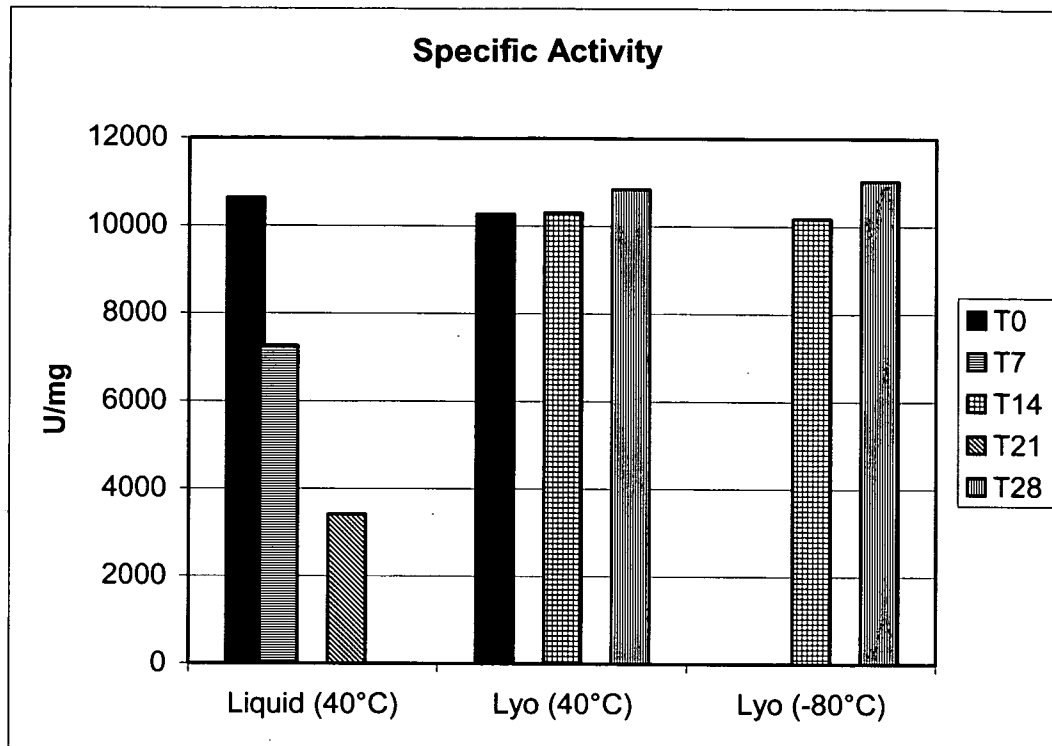
Figure 1

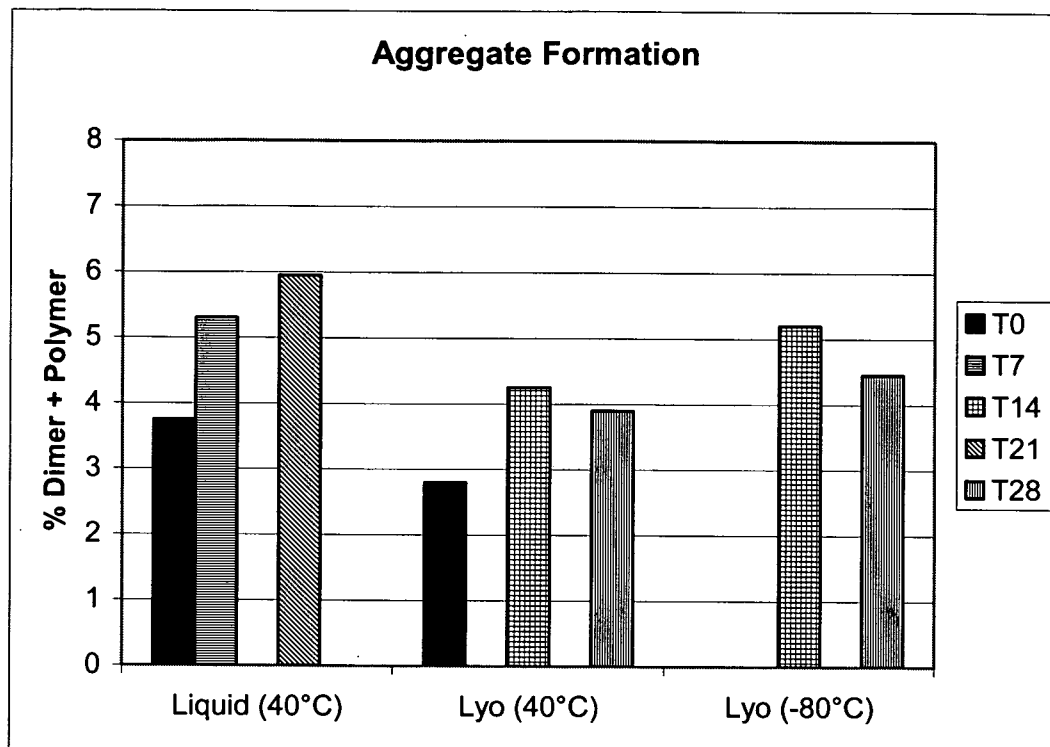
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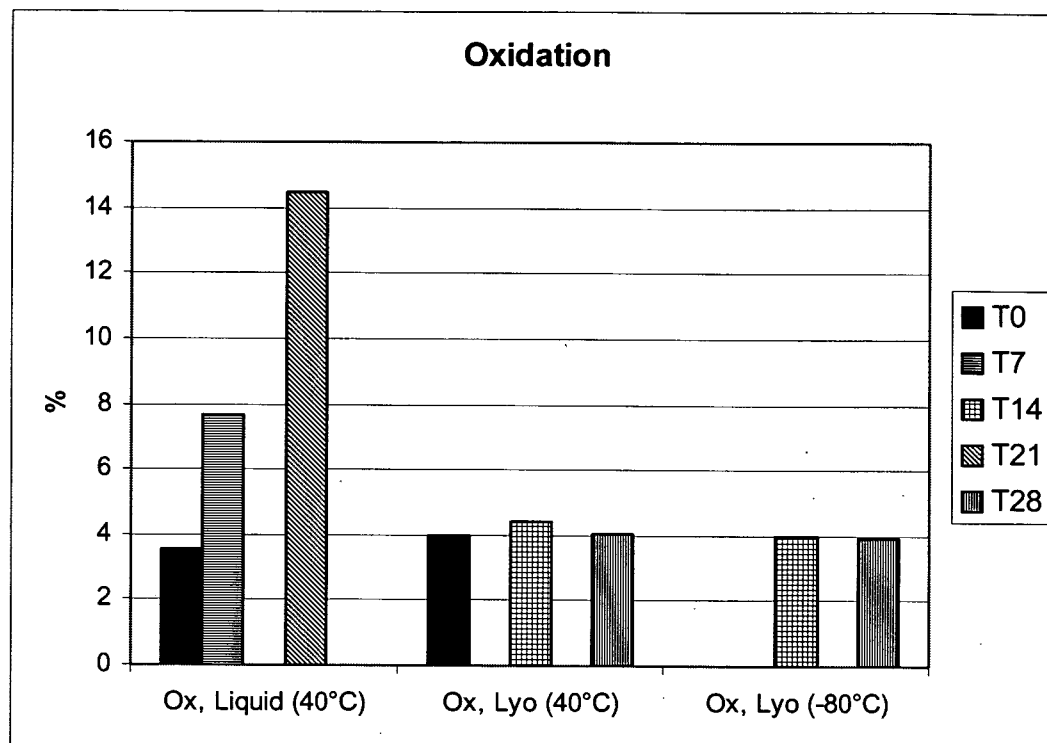
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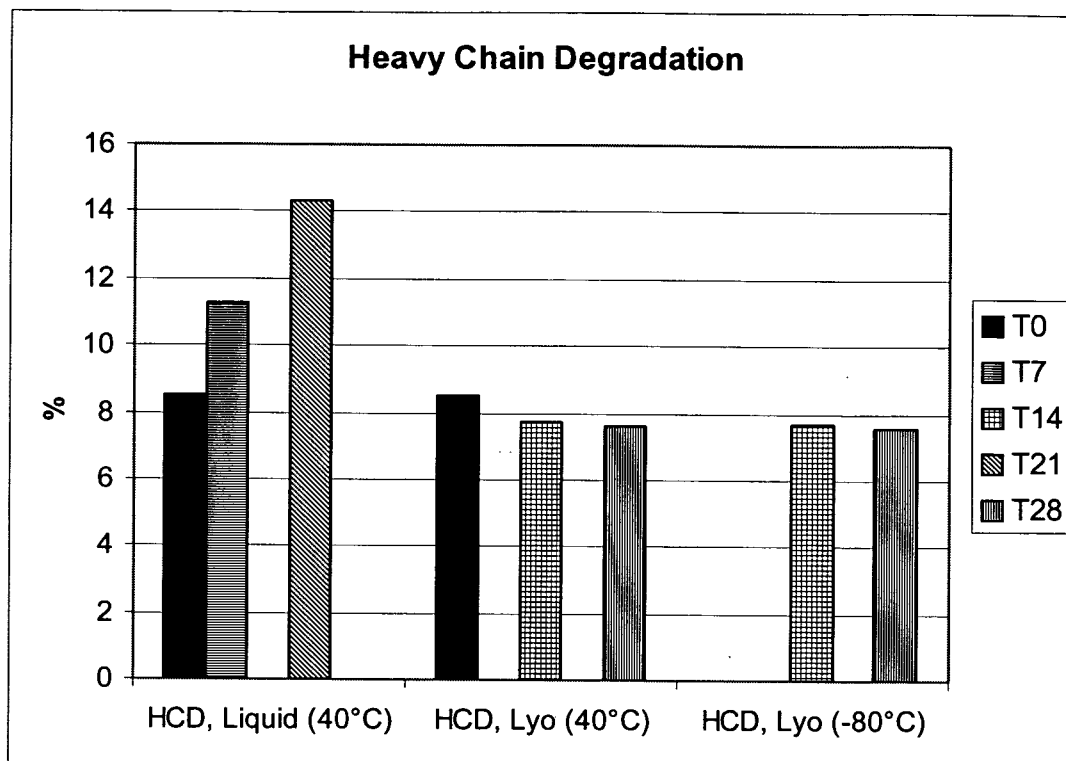
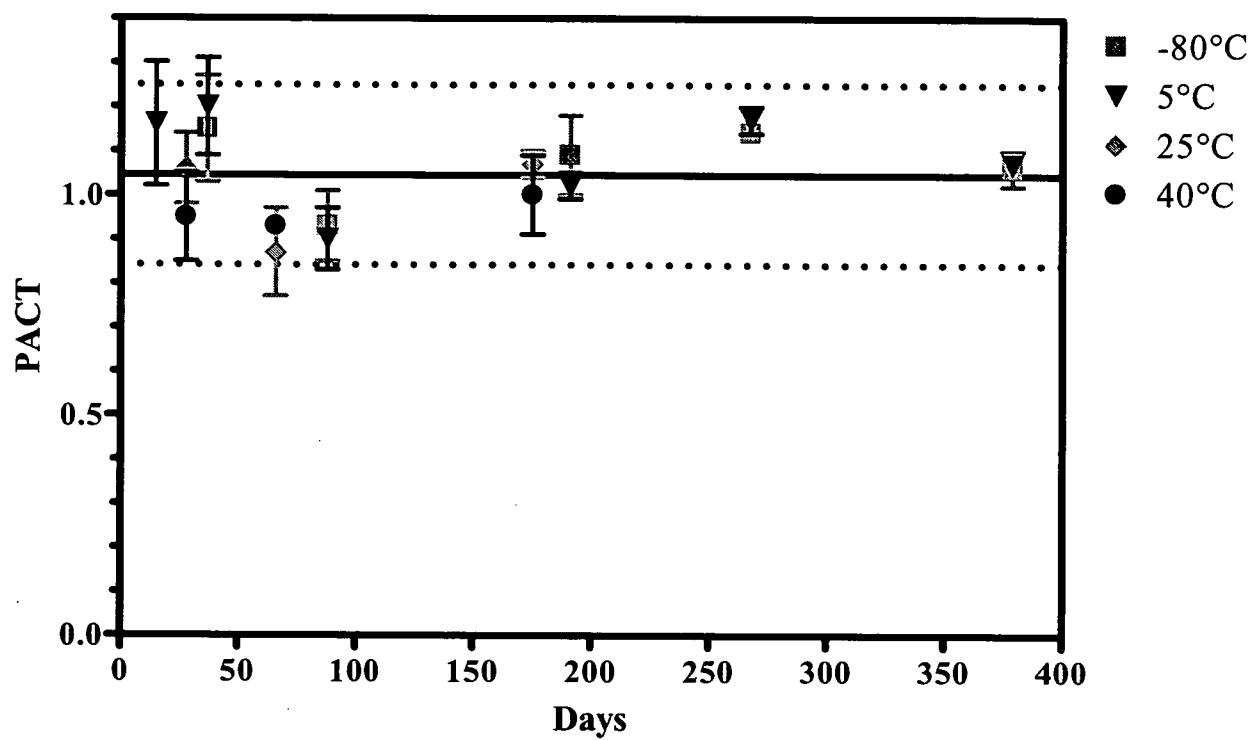
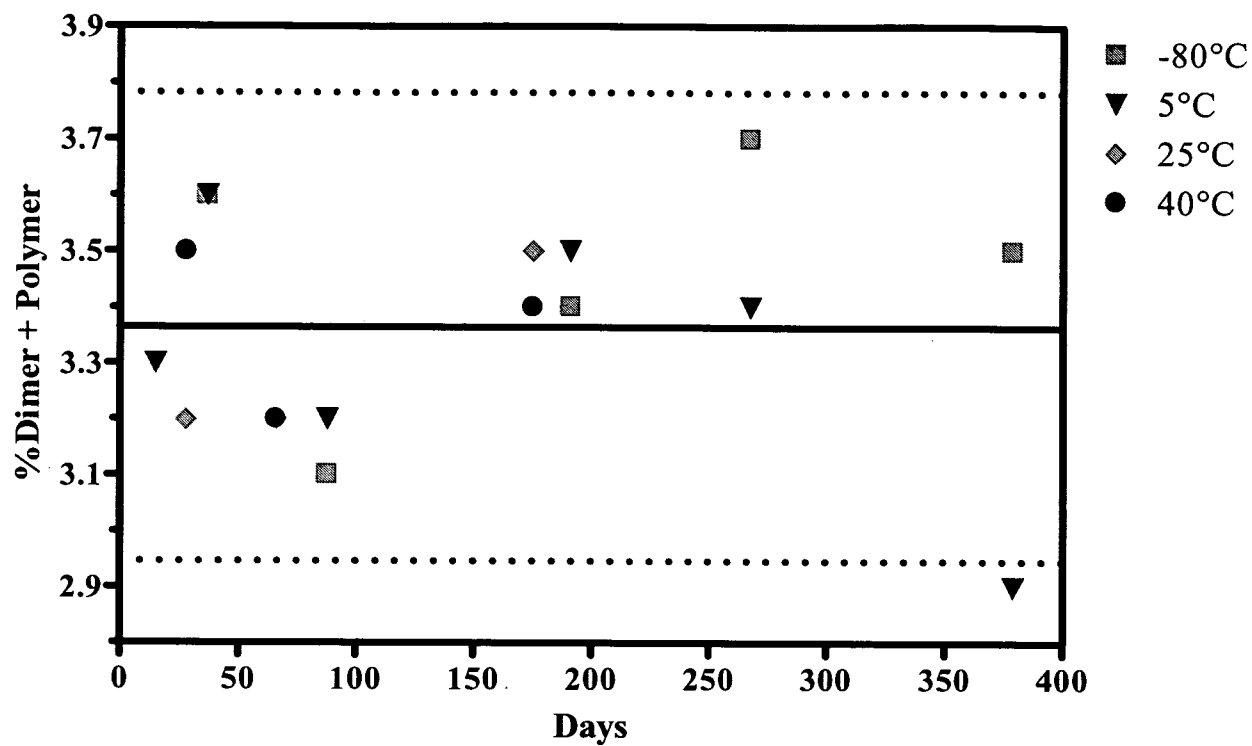
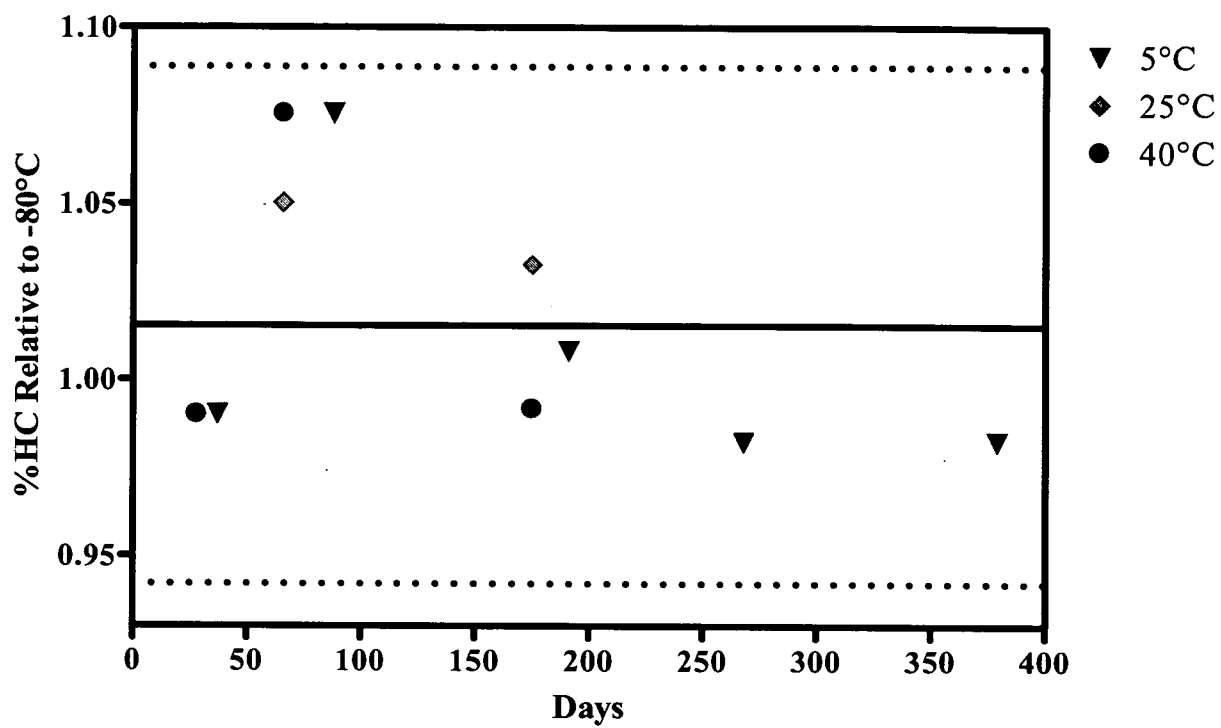
Figure 4

Figure 5

Relative potency (PACT assay)

Figure 6

Percent dimers + polymers

Figure 7

Heavy chain degradation (relative to samples stored at -80°C)

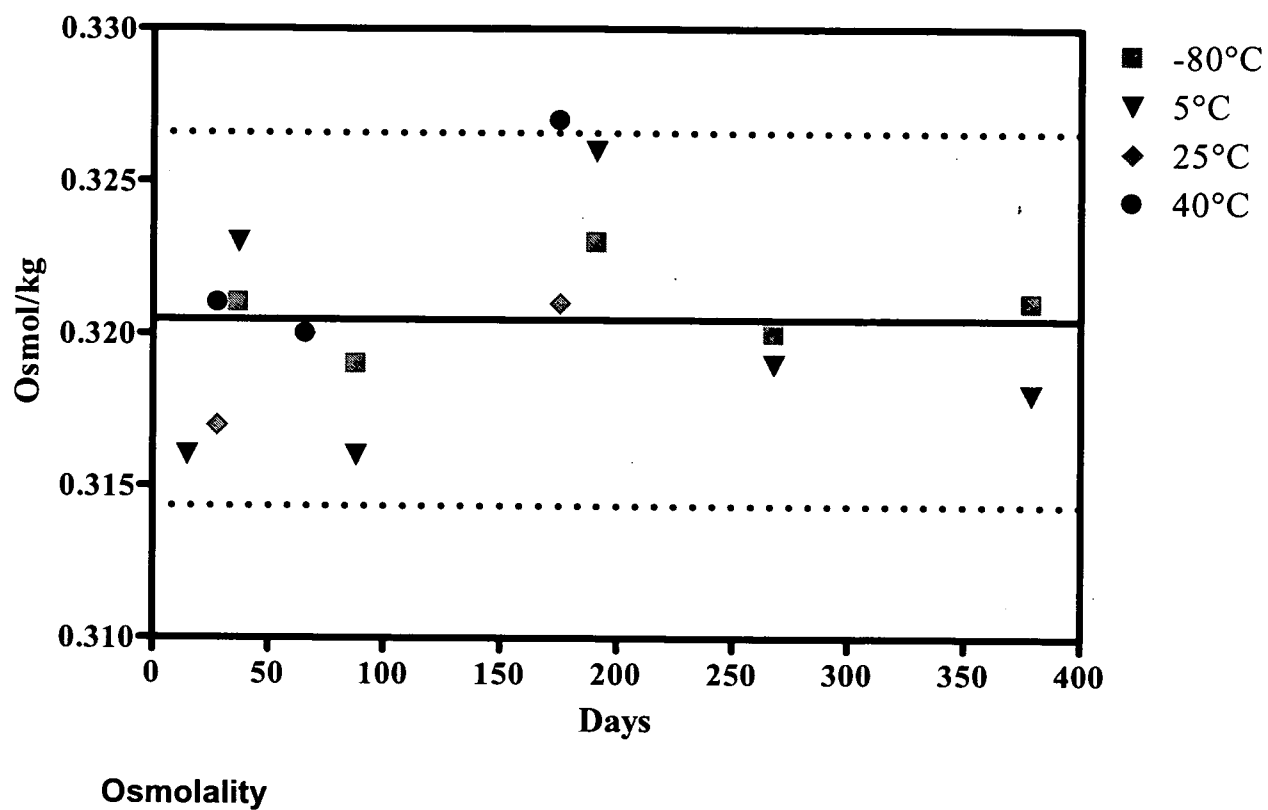
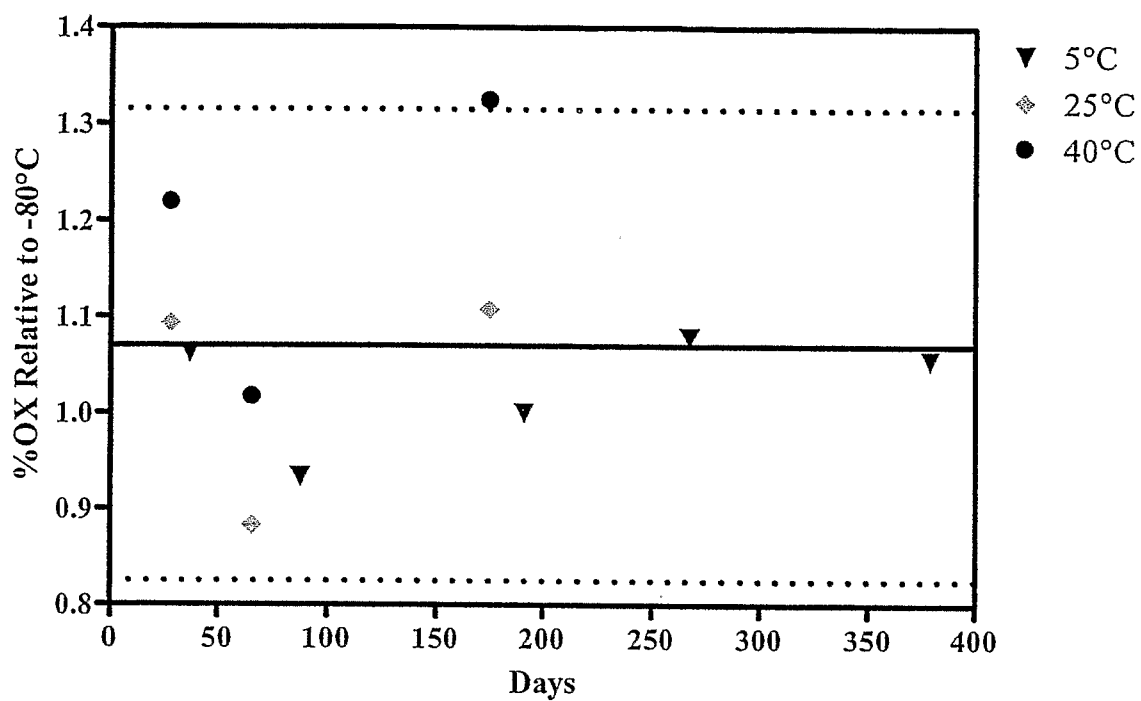
Figure 8

Figure 9



Oxidation (relative to samples stored at -80°C)