Stable pharmaceutical composition containing factor VIII

The invention relates to a new stable pharmaceutical composition containing factor VIII.

Factor VIII is a well-known plasma protein that is essential to the blood clotting process and is therefore used in the treatment of haemophilia.

Several forms of factor VIII have been used or are intended to be used as active principles for treating haemophilia. These include human factor VIII (like the active principles of Humate® P, Monoclate® P, Immunate® or Hemofil® M), recombinant human factor VIII (like r-VIII SQ which is described in PCT patent application WO 91/09122 (the active principle of ReFacto®) or the active principles of Kogenate® or Recombinate®), porcine factor VIII (which is the active principle of the product Hyate:C® sold by Ipsen, Inc., USA) or recombinant porcine factor VIII (e.g. a modified B-domainless form of porcine factor VIII like the one disclosed in patent application WO 01/68109 and identified as "POL1212" or the protein of SEQ. ID. NO. 38 of the same patent application).

Formulation stability has always been a problem for the pharmaceutical industry dealing with factor VIII pharmaceutical compositions.

Albumin has often been used to stabilise these formulations. However, despite its interesting stabilising effect, albumin presents the drawback of being expensive and also the risk to carry infectious species like prions. For these reasons, the pharmaceutical industry has been seeking in the recent years to replace albumin by other stabilising agents in factor VIII pharmaceutical compositions.

Several stable albumin-free pharmaceutical compositions are already known to the skilled artisan. For example:

- US patent No. 5,565,427 teaches a stabilised albumin-free solution with factor VIII:C activity containing factor VIII:C, an amino acid or one of its salts or homologues and a detergent (like polysorbate 80 or Tween® 80) or an organic polymer (like polyethyleneglycol).

- US patent No. 5,605,884 relates to a stable factor VIII composition comprising factor VIII and a high ionic strength media, which is preferably consisting of an aqueous

solution comprising a mixture of sodium chloride, calcium chloride and histidine as buffer ion.

- US patents Nos. 5,763,401 and 5,874,408 both disclose a stable albumin-free recombinant factor VIII composition comprising recombinant factor VIII, glycine, histidine, sucrose, sodium chloride and calcium chloride.

- US patent No. 5,962,650 teaches a stable albumin-free recombinant factor VIII composition which consists of an aqueous solution with a reduced concentration of oxygen comprising recombinant factor VIII, a calcium salt like calcium chloride and preferably an antioxidant, a non-ionic surfactant, sodium or potassium chloride, an amino acid and a mono- or disaccharide.

- US patent No. 5,972,885 relates to a pharmaceutical formulation for subcutaneous, intramuscular or intradermal administration which comprises highly concentrated (at least 1,000 IU/ml) recombinant factor VIII and, preferably, one or more elements selected from the group constituted (notably) by sodium or potassium chloride, calcium chloride, a non-ionic surfactant (e.g. a poloxamer), a mono- or disaccharide (preferably sucrose) and antioxidants (e.g. citric acid).

- PCT patent application WO 89/09784 discloses a method for the production of heat-stable factor VIII concentrate which comprises gel filtration of a buffer solution containing said factor VIII and tris(hydroxymethyl)methylamine, trisodium citrate, sodium chloride, sucrose and calcium chloride followed by freeze-drying of the concentrate obtained. The factor VIII thus produced is able to withstand temperatures of up to 80 °C for up to 72 hours.

- PCT patent application WO 94/07510 describes a factor VIII composition which is stabilised by a non-ionic surfactant (e.g. a poloxamer like polysorbate 80). Such a composition can also comprise one or more elements selected from the group constituted (notably) by sodium or potassium chloride, calcium chloride, an amino acid, a mono- or disaccharide such as sucrose,

The Applicant has now unexpectedly discovered that a solid pharmaceutical composition obtainable by lyophilisation of a solution devoid of amino acids comprising the following components:

(a) factor VIII;

(b) a surfactant;

(c) calcium chloride;

(d) sucrose;

(e) sodium chloride;

(f) trisodium citrate; and

(g) a buffer devoid of amino acids;

said pharmaceutical composition having a pH from 6 to 8 prior to lyophilisation and after reconstitution in water for injection, also shows stability over time.

By factor VIII is meant in the present application human factor VIII, recombinant human factor VIII, porcine factor VIII, recombinant porcine factor VIII or more generally any other recombinant factor VIII that can be used to replace them.

Preferably, the factor VIII comprised in compositions according to the invention, will be chosen from porcine factor VIII or recombinant porcine factor VIII. Still more preferably, the factor VIII comprised in compositions according to the invention, will be recombinant porcine factor VIII, especially a modified B-domainless form of porcine factor VIII like the one disclosed in patent application WO 01/68109, i.e. the modified porcine factor VIII having the amino acid sequence SEQ. ID. NO. 1 hereafter:

SEQ. ID. NO. 1:

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Met Gln Leu Glu Leu Ser Thr Cys Val Phe Leu Cys Leu Leu Pro Leu
 1               5                  10                  15

Gly Phe Ser Ala Ile Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser
            20                  25                  30

Trp Asp Tyr Arg Gln Ser Glu Leu Leu Arg Glu Leu His Val Asp Thr
        35                  40                  45

Arg Phe Pro Ala Thr Ala Pro Gly Ala Leu Pro Leu Gly Pro Ser Val
    50                  55                  60

Leu Tyr Lys Lys Thr Val Phe Val Glu Phe Thr Asp Gln Leu Phe Ser
65                  70                  75                  80

Val Ala Arg Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile
                85                  90                  95

Gln Ala Glu Val Tyr Asp Thr Val Val Val Thr Leu Lys Asn Met Ala
            100                 105                 110

Ser His Pro Val Ser Leu His Ala Val Gly Val Ser Phe Trp Lys Ser
        115                 120                 125

Ser Glu Gly Ala Glu Tyr Glu Asp His Thr Ser Gln Arg Glu Lys Glu
    130                 135                 140
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Asp Asp Lys Val Leu Pro Gly Lys Ser Gln Thr Tyr Val Trp Gln Val
145                 150                 155                 160

Leu Lys Glu Asn Gly Pro Thr Ala Ser Asp Pro Pro Cys Leu Thr Tyr
                165                 170                 175

Ser Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu
            180                 185                 190

Ile Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Thr Arg Glu Arg
        195                 200                 205

Thr Gln Asn Leu His Glu Phe Val Leu Leu Phe Ala Val Phe Asp Glu
    210                 215                 220

Gly Lys Ser Trp His Ser Ala Arg Asn Asp Ser Trp Thr Arg Ala Met
225                 230                 235                 240

Asp Pro Ala Pro Ala Arg Ala Gln Pro Ala Met His Thr Val Asn Gly
                245                 250                 255

Tyr Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Lys Lys Ser
            260                 265                 270

Val Tyr Trp His Val Ile Gly Met Gly Thr Ser Pro Glu Val His Ser
        275                 280                 285

Ile Phe Leu Glu Gly His Thr Phe Leu Val Arg His His Arg Gln Ala
    290                 295                 300

Ser Leu Glu Ile Ser Pro Leu Thr Phe Leu Thr Ala Gln Thr Phe Leu
305                 310                 315                 320

Met Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His His
                325                 330                 335

His Gly Gly Met Glu Ala His Val Arg Val Glu Ser Cys Ala Glu Glu
            340                 345                 350

Pro Gln Leu Arg Arg Lys Ala Asp Glu Glu Glu Asp Tyr Asp Asp Asn
        355                 360                 365

Leu Tyr Asp Ser Asp Met Asp Val Val Arg Leu Asp Gly Asp Asp Val
    370                 375                 380

Ser Pro Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
385                 390                 395                 400

Trp Val His Tyr Ile Ser Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro
                405                 410                 415

Ala Val Pro Ser Pro Ser Asp Arg Ser Tyr Lys Ser Leu Tyr Leu Asn
            420                 425                 430

Ser Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Ala Arg Phe Val
        435                 440                 445

Ala Tyr Thr Asp Val Thr Phe Lys Thr Arg Lys Ala Ile Pro Tyr Glu
    450                 455                 460

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
465                 470                 475                 480

Leu Ile Ile Phe Lys Asn Lys Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
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485 490 495

His Gly Ile Thr Asp Val Ser Ala Leu His Pro Gly Arg Leu Leu Lys
500 505 510

Gly Trp Lys His Leu Lys Asp Met Pro Ile Leu Pro Gly Glu Thr Phe
515 520 525

Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
530 535 540

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Ser Ile Asn Leu Glu Lys
545 550 555 560

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu
565 570 575

Ser Val Asp Gln Arg Gly Asn Gln Met Met Ser Asp Lys Arg Asn Val
580 585 590

Ile Leu Phe Ser Val Phe Asp Glu Asn Gln Ser Trp Tyr Leu Ala Glu
595 600 605

Asn Ile Gln Arg Phe Leu Pro Asn Pro Asp Gly Leu Gln Pro Gln Asp
610 615 620

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
625 630 635 640

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp
645 650 655

Tyr Ile Leu Ser Val Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe
660 665 670

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr
675 680 685

Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro
690 695 700

Gly Leu Trp Val Leu Gly Cys His Asn Ser Asp Leu Arg Asn Arg Gly
705 710 715 720

Met Thr Ala Leu Leu Lys Val Tyr Ser Cys Asp Arg Asp Ile Gly Asp
725 730 735

Tyr Tyr Asp Asn Thr Tyr Glu Asp Ile Pro Gly Phe Leu Leu Ser Gly
740 745 750

Lys Asn Val Ile Glu Pro Arg Ser Phe Ala Gln Asn Ser Arg Pro Pro
755 760 765

Ser Ala Ser Ala Pro Lys Pro Pro Val Leu Arg Arg His Gln Arg Asp
770 775 780

Ile Ser Leu Pro Thr Phe Gln Pro Glu Glu Asp Lys Met Asp Tyr Asp
785 790 795 800

Asp Ile Phe Ser Thr Glu Thr Lys Gly Glu Asp Phe Asp Ile Tyr Gly
805 810 815

Glu Asp Glu Asn Gln Asp Pro Arg Ser Phe Gln Lys Arg Thr Arg His
820 825 830

Tyr Phe Ile Ala Ala Val Glu Gln Leu Trp Asp Tyr Gly Met Ser Glu
835 840 845

Ser Pro Arg Ala Leu Arg Asn Arg Ala Gln Asn Gly Glu Val Pro Arg
850 855 860

Phe Lys Lys Val Val Phe Arg Glu Phe Ala Asp Gly Ser Phe Thr Gln
865 870 875 880

Pro Ser Tyr Arg Gly Glu Leu Asn Lys His Leu Gly Leu Leu Gly Pro
885 890 895

Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met Val Thr Phe Lys Asn
900 905 910

Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr Pro
915 920 925

Asp Asp Gln Glu Gln Gly Ala Glu Pro Arg His Asn Phe Val Gln Pro
930 935 940

Asn Glu Thr Arg Thr Tyr Phe Trp Lys Val Gln His His Met Ala Pro
945 950 955 960

Thr Glu Asp Glu Phe Asp Cys Lys Ala Trp Ala Tyr Phe Ser Asp Val
965 970 975

Asp Leu Glu Lys Asp Val His Ser Gly Leu Ile Gly Pro Leu Leu Ile
980 985 990

Cys Arg Ala Asn Thr Leu Asn Ala Ala His Gly Arg Gln Val Thr Val
995 1000 1005

Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe Asp Glu Thr Lys Ser Trp
1010 1015 1020

Tyr Phe Thr Glu Asn Val Glu Arg Asn Cys Arg Ala Pro Cys His Leu
1025 1030 1035 1040

Gln Met Glu Asp Pro Thr Leu Lys Glu Asn Tyr Arg Phe His Ala Ile
1045 1050 1055

Asn Gly Tyr Val Met Asp Thr Leu Pro Gly Leu Val Met Ala Gln Asn
1060 1065 1070

Gln Arg Ile Arg Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn Ile
1075 1080 1085

His Ser Ile His Phe Ser Gly His Val Phe Ser Val Arg Lys Lys Glu
1090 1095 1100

Glu Tyr Lys Met Ala Val Tyr Asn Leu Tyr Pro Gly Val Phe Glu Thr
1105 1110 1115 1120

Val Glu Met Leu Pro Ser Lys Val Gly Ile Trp Arg Ile Glu Cys Leu
1125 1130 1135

Ile Gly Glu His Leu Gln Ala Gly Met Ser Thr Thr Phe Leu Val Tyr
1140 1145 1150

Ser Lys Glu Cys Gln Ala Pro Leu Gly Met Ala Ser Gly Arg Ile Arg
1155 1160 1165

Asp Phe Gln Ile Thr Ala Ser Gly Gln Tyr Gly Gln Trp Ala Pro Lys
1170 1175 1180

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Leu Ala Arg Leu His Tyr Ser Gly Ser Ile Asn Ala Trp Ser Thr Lys
1185                1190                1195                1200

Asp Pro His Ser Trp Ile Lys Val Asp Leu Leu Ala Pro Met Ile Ile
                1205                1210                1215

His Gly Ile Met Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser Leu Tyr
            1220                1225                1230

Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly Arg Asn Trp Gln
        1235                1240                1245

Ser Tyr Arg Gly Asn Ser Thr Gly Thr Leu Met Val Phe Phe Gly Asn
    1250                1255                1260

Val Asp Ala Ser Gly Ile Lys His Asn Ile Phe Asn Pro Pro Ile Val
1265                1270                1275                1280

Ala Arg Tyr Ile Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser Thr
                1285                1290                1295

Leu Arg Met Glu Leu Met Gly Cys Asp Leu Asn Ser Cys Ser Met Pro
            1300                1305                1310

Leu Gly Met Gln Asn Lys Ala Ile Ser Asp Ser Gln Ile Thr Ala Ser
        1315                1320                1325

Ser His Leu Ser Asn Ile Phe Ala Thr Trp Ser Pro Ser Gln Ala Arg
    1330                1335                1340

Leu His Leu Gln Gly Arg Thr Asn Ala Trp Arg Pro Arg Val Ser Ser
1345                1350                1355                1360

Ala Glu Glu Trp Leu Gln Val Asp Leu Gln Lys Thr Val Lys Val Thr
                1365                1370                1375

Gly Ile Thr Thr Gln Gly Val Lys Ser Leu Leu Ser Ser Met Tyr Val
            1380                1385                1390

Lys Glu Phe Leu Val Ser Ser Ser Gln Asp Gly Arg Arg Trp Thr Leu
        1395                1400                1405

Phe Leu Gln Asp Gly His Thr Lys Val Phe Gln Gly Asn Gln Asp Ser
    1410                1415                1420

Ser Thr Pro Val Val Asn Ala Leu Asp Pro Pro Leu Phe Thr Arg Tyr
1425                1430                1435                1440

Leu Arg Ile His Pro Thr Ser Trp Ala Gln His Ile Ala Leu Arg Leu
                1445                1450                1455

Glu Val Leu Gly Cys Glu Ala Gln Asp Leu Tyr
            1460                1465
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Preferably, the surfactant will be a non-ionic surfactant. Non-ionic surfactants include notably polysorbates and block copolymers like poloxamers (i.e. copolymers of polyethylene and propylene glycol). According to a preferred variant of the invention, the surfactant will be a polysorbate. More preferably, a polysorbate included in a composition according to the instant invention will have a mean polymerisation degree

of from 20 to 100 monomer units (preferably about 80), and may for example be polysorbate 80. Preferably also, the polysorbate should be vegetable-derived.

Preferably, the buffer devoid of amino acids will be tris(hydroxymethyl)methylamine (hereafter abridged "tris").

Preferably also, the pH of the pharmaceutical composition prior to lyophilisation and after reconstitution in water for injection will be from 6.5 to 7.5, and more preferably about 7.0.

Preferably, a solid composition according to the invention will be such that it may be obtained by lyophilisation of a solution devoid of amino acids that comprises:

(a) a concentration of factor VIII ranging from 50 to 10,000 international units/ml for human or recombinant human factor VIII or from 50 to 10,000 porcine units/ml for porcine or recombinant porcine factor VIII;

(b) a concentration of surfactant ranging from above critical micellar concentration to 1% v/v;

(c) a concentration of calcium chloride ranging from 0.5 to 10 mM;

(d) a concentration of sucrose ranging from 5 to 50 mM;

(e) a concentration of sodium chloride ranging from 0.15 to 0.5 M;

(f) a concentration of trisodium citrate ranging from 1 to 50 mM; and

(g) a concentration of buffer devoid of amino acids ranging from 1 to 50 mM.

For evaluating the activity in terms of international factor VIII units, the product to be tested is assayed against a Concentrate Standard, such as the United Kingdom standard NIBSC 95/608 (NIBSC for National Institute of Biological Standards and Control).

By porcine unit of factor VIII is meant the United Kingdom national standard unit held by United Kingdom's NIBSC. For evaluating the activity in terms of porcine factor VIII units, the product to be tested is assayed against the UK national porcine standard NIBSC 86/514. Concerning recombinant porcine factor VIII, it should be understood that 1 unit of activity for recombinant porcine factor VIII is equivalent to 1 unit of activity for porcine factor VIII.

More preferably, a solid composition according to the invention will be such that it may be obtained by lyophilisation of a solution devoid of amino acids that comprises at least one of the following characteristics:

- a concentration of factor VIII ranging from 100 to 5,000 international units/ml for human or recombinant human factor VIII or from 100 to 5,000 porcine units/ml for porcine or recombinant porcine factor VIII;
- a concentration of surfactant ranging from 0.002% to 0.04% v/v;
- a concentration of calcium chloride ranging from 1 to 5 mM;
- a concentration of sucrose ranging from 5 to 25 mM;
- a concentration of sodium chloride ranging from 0.2 to 0.4 M;
- a concentration of trisodium citrate ranging from 1 to 20 mM; or
- a concentration of buffer devoid of amino acids ranging from 1 to 20 mM.

Even more preferably, a solid composition according to the invention will be such that it may be obtained by lyophilisation of a solution devoid of amino acids that comprises at least one of the following characteristics:

- a concentration of factor VIII ranging from 200 to 2,000 international units/ml (and notably about 1,000 international units/ml) for human or recombinant human factor VIII or from 200 to 2,000 porcine units/ml (and notably about 1,000 porcine units/ml) for porcine or recombinant porcine factor VIII;
- a concentration of surfactant ranging from about 0.002% to 0.02% v/v (and notably about 0.01% v/v);
- a concentration of calcium chloride ranging from 1 to 3 mM (and notably about 2 mM);
- a concentration of sucrose ranging from 5 to 15 mM (and notably about 11.7 mM) ;
- a concentration of sodium chloride ranging from 0.25 to 0.35 M (and notably about 0.3 M);
- a concentration of trisodium citrate ranging from 1 to 20 mM (and notably about 10 mM); or

- a concentration of buffer devoid of amino acids ranging from 5 to 15 mM (and notably about 10 mM).

The solid factor VIII compositions according to the invention may be prepared by lyophilising a solution comprising the appropriate quantities of the components identified above as (a), (b), (c), (d), (e), (f) and (g) according to standard manufacturing procedures (sterile conditions, etc.).

Stability of the composition over a certain period may be determined, for example, by the method described hereunder in the part entitled "Analytical methods", or by any other method found appropriate by the skilled artisan.

A composition according to the invention is considered stable during a certain period of time if 70% to 130% (and preferably 80% to 120%) of the initial factor VIII activity, as evaluated using the method disclosed the part entitled "*Analytical methods*" hereafter, is maintained over said period of time.

Preferably, the solid compositions of this invention will be stable for at least 6 or 12 months when kept at a temperature of 2 to 8 °C. More preferably, they will be stable for at least 6 or 12 months when kept at a temperature of 30 to 32 °C.

The solid factor VIII compositions according to the invention may be diluted with sterile water optionally containing sodium chloride, and the resulting liquid pharmaceutical composition may then be directly injected into a patient in need thereof. The resulting liquid pharmaceutical composition, as well as liquid pharmaceutical compositions obtainable by dilution of solid factor VIII compositions according to the invention with sterile water optionally containing sodium chloride, are also part of this invention.

Methods of treatment of haemophilia comprising the administration of a liquid composition according to the invention to a patient in need thereof are also within the scope of this invention. The administration mode contemplated for liquid compositions according to the instant invention will preferably be intravenous administration. The dose of composition according to the instant invention which is to be administered will be determined by the treating physician or veterinarian, taking into account the severity of the disease for each patient.

The term "about" refers to an interval around the considered value. As used in this patent application, "about X" means an interval from X minus 10% of X to X plus 10% of X, and preferably an interval from X minus 5% of X to X plus 5% of X.

Unless they are defined differently, all the technical and scientific terms used here have the same meaning as that usually understood by an ordinary specialist in the field to which this invention belongs. Similarly, all publications, patent applications, all patents and all other references mentioned here are incorporated by way of reference.

The following examples are presented to illustrate the above and must in no case be considered as a limit to the scope of the invention.

EXAMPLES

Example 1:

A solution in 0.5 ml sterile water containing the following components is prepared:

Modified porcine factor VIII of sequence SEQ. ID. NO. 1	800 porcine units/ml
Vegetable derived polysorbate 80	0.01% v/v
Calcium chloride	2 mM
Sucrose	11.7 mM
Sodium chloride	0.3 M
Tri sodium citrate	10 mM
Tris buffer	10 mM
pH	7.0

The mixture is lyophilised in a sterilised vial which is then sealed. The solid composition obtained has been tested and shown to be stable at a temperature of 2 to 8 °C for at least 18 months and at 30 to 32°C for at least six months when tested by factor VIII activity. There was no indication of high molecular weight component formation as assessed by Size Exclusion HPLC (SEC HPLC) or fragments as assessed by SDS PAGE.

The lyophilised mixture obtained would typically be reconstituted with 1.0 ml sterile water before injection into a patient.

Example 2:

A solution in 1.0 ml sterile water containing the following components is prepared:

Modified porcine factor VIII of sequence SEQ. ID. NO. 1	400 porcine units/ml

Vegetable derived polysorbate 80	0.002% v/v
Calcium chloride	2 mM
Sucrose	11.7 mM
Sodium chloride	0.3 M
Tri sodium citrate	10 mM
Tris buffer	10 mM
pH	7.0

The mixture is lyophilised in a sterilised vial which is then sealed.

The lyophilised mixture obtained would typically be reconstituted with 2.0 ml sterile water before injection into a patient.

Example 3:

A solution in 0.5 ml sterile water containing the following components is prepared:

Plasma-derived porcine factor VIII	100 porcine units/ml
Vegetable derived polysorbate 80	0.01% v/v
Calcium chloride	2 mM
Sucrose	11.7 mM
Sodium chloride	0.3 M
Tri sodium citrate	10 mM
Tris buffer	10 mM
pH	7.0

The mixture is lyophilised in a sterilised vial which is then sealed.

The lyophilised mixture obtained would typically be reconstituted with 1.0 ml sterile water before injection into a patient.

ANALYTICAL METHODS

Chromogenic assay

The factor VIII activity is determined by a modified chromogenic assay (Technochrom FVIII:C Reagent Kit, Technoclone). The generation of activated factor X by factor IX is stimulated by factor VIII which acts as a cofactor in the reaction. The release of

p-nitroaniline from the chromogenic substrate is catalysed by activated factor X. The amount of *p*-nitroaniline which is released is measured photometrically at 405 nm and the assay gives a linear correlation between the amount of *p*-nitroaniline generated and the FVIII content.

SEC HPLC

Soluble high molecular weight components and fragments were determined by gel filtration performed on a HPLC instrument using a TosoHaas TSK G3000 SWXL, 0.78 x 30 cm pre-packed column with a fluorescence detector (Waters LC Module 1 plus). Excitation wavelength 280 nm and emission wavelength 340 nm. Evaluation of the results were performed by integration of the peak areas.

SDS PAGE assay

SDS PAGE (polyacylamide gel electrophoresis using a flatbed electrophoresis system (Multiphor II LKB) and pre cast 7.5% gels (EXCELGEL SDS, Pharmacia) was used to determine any breakdown products of the FVIII molecule. Protein bands were visualised by Coomassie blue staining.

Stability assay

Stability can be assayed by performing the above described assays at different times on a sample of the same composition held at the temperature chosen (which may be around + 4 °C or + 31 °C). Once its factor VIII activity will have dropped of more than 30%, the composition will be considered to have lost its stability.

CLAIMS

1. A solid pharmaceutical composition obtainable by lyophilisation of a solution devoid of amino acids comprising

(a) factor VIII ;

(b) a surfactant;

(c) calcium chloride;

(d) sucrose;

(e) sodium chloride;

(f) trisodium citrate; and

(g) a buffer devoid of amino acids;

having a pH from 6 to 8 prior to lyophilisation and after reconstitution in water for injection.

2. A solid pharmaceutical composition according to claim 1 characterised in that the factor VIII is chosen from porcine factor VIII or recombinant porcine factor VIII.

3. A solid pharmaceutical composition according to claim 2 characterised in that the factor VIII is recombinant porcine factor VIII.

4. A solid pharmaceutical composition according to claim 2 characterised in that the recombinant porcine factor VIII has the amino acid sequence SEQ. ID. NO. 1.

5. A solid pharmaceutical composition according to one of claims 1 to 4 characterised in that the surfactant is a polysorbate.

6. A solid pharmaceutical composition according to claim 5 characterised in that the surfactant is a polysorbate 80.

7. A solid pharmaceutical composition according to one of claims 1 to 6 characterised in that the buffer devoid of amino acids is tris(hydroxymethyl)methylamine.

8. A solid pharmaceutical composition according to one of claims 1 to 7 which, prior to lyophilisation and after reconstitution in water for injection, has a pH from 6.5 to 7.5.

9. A solid pharmaceutical composition according to one of claims 1 to 8, which may be obtained by lyophilisation of a solution devoid of amino acids that comprises:

(a) a concentration of factor VIII ranging from 50 to 10,000 international units/ml for human or recombinant human factor VIII or from 50 to 10,000 porcine units/ml for porcine or recombinant porcine factor VIII;

(b) a concentration of surfactant ranging from above critical micellar concentration to 1% v/v;

(c) a concentration of calcium chloride ranging from 0.5 to 10 mM;

(d) a concentration of sucrose ranging from 5 to 50 mM;

(e) a concentration of sodium chloride ranging from 0.15 to 0.5 M;

(f) a concentration of trisodium citrate ranging from 1 to 50 mM; and

(g) a concentration of a buffer devoid of amino acids ranging from 1 to 50 mM.

10. A liquid pharmaceutical composition obtainable after dilution of a solid pharmaceutical composition according to one of claims 1 to 9 with sterile water optionally containing sodium chloride.