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DESCRIPTION

Description

FIELD OF INVENTION

[0001] The present invention relates to the field of medicine. More particularly, the invention relates to a pharmaceutical composition comprising plasminogen, and therapeutic uses thereof.

BACKGROUND OF INVENTION

[0002] Plasminogen is a zymogen of plasmin as shown in Figure 1A. The amino acid sequence of the human plasminogen precursor is depicted in Figure 1B. Human plasminogen contains 791 amino acids (precursor = 810 amino acids) with a molecular weight of about 90 kDa and a pI of approximately 7.0, although differential glycosylation and/or removal of the N-terminal activation peptide can result in a pI range of 6.2 to 8.0. It is a single-chain protein with 24 intra-chain disulfide bridges, 5 kringle domains (involved in the binding to fibrin and to the inhibitor α 2-antiplasmin), a serine protease domain (P), and an activation peptide (AP) consisting of the first 77 amino acids. There is one N-linked glycosylation site and one O-linked site, although a second O-linked site has been identified (Goldberg, 2006). Approximately 70% of the Plasminogen in circulation contains only O-linked glycosylation while the rest contains both N- and O-linked sugars.

[0003] Native Plasminogen is produced in two main forms, Glu-Plasminogen (Glu-Pg) and Lys-Plasminogen (Lys-Pg), named for the N-terminal amino acid of either glutamic acid or lysine. Glu-Pg is composed of the entire amino acid sequence designated by the gene sequence (excluding the activation peptide), while Lys-Pg is the result of a cleavage of the Glu-Pg between Lys-77 and Lys-78 (underlined in Figure 1B). The circulating half-life of Lys-Pg is considerably shorter than Glu-Pg (2-2.5 days for Glu-Pg, 0.8 days for Lys-Pg). Glu-Pg is the dominant form of Pg present in plasma with very little Lys-Pg detected in the circulation (Violand, B.N., Byrne, R., Castellino F.J. (1978) The effect of α , ω -Amino Acids on Human Plasminogen Structure and Activation. J Biol Chem. 253 (15): 5395-5401; Collen D, Ong EB, Johnson AJ. (1975) Human Plasminogen: In Vitro and In Vivo Evidence for the Biological Integrity of NH₂-Terminal Glutamic Acid Plasminogen. Thrombosis Research. 7 (4):515-529).

[0004] Plasminogen is synthesized in the liver and secreted into plasma. Plasminogen is distributed throughout the body and when conditions are present for activation, the

plasminogen pro-enzyme is converted to the active enzyme, plasmin, by tissue-type plasminogen activator (t-PA) or by urokinase plasminogen activator (u-PA). Plasmin then degrades fibrin and converts latent matrix metalloproteinases (pro-MMPs) into active MMPs, which in turn further degrade extracellular matrix (ECM) as part of the tissue healing/remodeling process. Plasminogen activation mediated by t-PA is primarily involved in fibrin homeostasis, while plasmin generation via u-PA, forming a complex with its receptor u-PAR, plays a role in tissue remodeling.

[0005] Plasmin is or was investigated for its potential use for the clearance of thrombotic occlusions in artificial devices and hemodialysis grafts, and for the treatment of posterior vitreous detachment (PVD) (US 6,969,515; US 2010/0104551).

[0006] Plasminogen is investigated for its use in therapeutic indications such as wound healing, healing of a tympanic membrane perforation, healing of periodontal wound, infectious disease, oral health, diabetic ulcer, thrombolysis indications, such as coronary thrombosis, reperfusion injury to tissue, ischemia, infarction, brain edema, improvement of microcirculation, and modulation of complement pathway (US 8,637,010; US 8,679,482; US 8,318,661; WO 95/12407; EP 0,631,786). As yet, no plasminogen is currently on the pharmaceutical market as a drug.

[0007] Historically, Lys-Pg was pharmaceutically commercialized for a period of time for hematologic purposes and has not been scientifically or medically used since 2000. A formulation of Lys-Pg is described in Schott et al., 1998, *The New England Journal of Medicine*, Vol. 339, No. 23, pp. 1679-1686). While available Lys-Pg was clinically used and investigated for the treatment of ligneous conjunctivitis, a clinical manifestation of the underlying condition hypoplasminogenemia (plasminogen deficiency type I). Hence systemic administration of Lys-Pg concentrates has been tested. Kraft et al. (Kraft J, Lieb W, Zeitler P, Schuster V. (2000) Ligneous conjunctivitis in a girl with severe type I plasminogen deficiency. *Graefes Arch Clin Exp Ophthalmol.* 238(9):797-800) reported that daily infusion of Lys-Pg in a child with severe hypoplasminogenemia resulted in partial resolution of the conjunctival pseudo membranes. Schott et al. (1998) reported that, in a 6-month-old child, treatment with Lys-Pg preparation as a continuous infusion and later as daily bolus injections led to complete regression of the ligneous conjunctivitis within 4 weeks and normalized hyperviscous secretions in the respiratory tract as well as skin wound healing. Schuster et al (Schuster V, Hugle B, Tefs K (2007) Plasminogen deficiency. *J Thromb Haemost* 5(12):2315-2322) reported that plasminogen levels in patients with homozygous or compound heterozygous (the presence of two different mutant alleles at a particular gene locus, one on each chromosome of a pair) hypoplasminogenemia ranged from <1 to 9 mg/dL for plasminogen antigen plasma levels and <1% to 51% for functional plasminogen activity. It is important to note that the majority of these patients have some residual plasminogen activity levels. Thus, plasminogen replacement is expected to be effective, as it is an endogenous protein and is not expected to have immunogenicity or fibrinolytic activity concerns. Although systemic or topical plasminogen concentrates have been clearly documented as effective therapy leading to resolution and halts re-formation of the lesions (Watts P, Suresh P, Mezer E, Ells A, Albisetti M, Bajzar L,

Marzinotto V, Andrew M, Massicotte P, Rootman D (2002) Effective treatment of ligneous conjunctivitis with topical plasminogen. *Am J Ophthalmol* 133(4):451-455, Heidemann DG, Williams GA, Hartzler M, Ohanian A, Citron ME (2003) Treatment of ligneous conjunctivitis with topical plasmin and topical plasminogen. *Cornea* 22(8):760-762; and Schott, 1998), no purified plasminogen product for topical or for systemic therapy is commercially available.

[0008] Only very recently, clinical trials were undertaken to utilize Glu-Pg for the treatment of the type I plasminogen deficiency (Clinical Trials.gov Identifier: NCT02312180) and one of its clinical manifestation ligneous conjunctivitis utilizing a localized eye drop of Glu-Pg (ClinicalTrials.gov Identifier: NCT01554956).

[0009] Proteins may be stabilized either by changing their structural characteristics (internally) and/or by controlling the components in contact with them (externally). Some proteins have raised particular challenges with respect to handling and behaviour in pharmaceutical formulations due to their physico-chemical characteristics which often unfortunately leads to structural instability. They can actually undergo various types of degradations as exemplified by the following: 1) chemical processes leading to the formation of related impurities, which may involve hydrolysis, oxidation reactions, deamidation or structural rearrangements such as iso-asp or intramolecular truncations or 2) a physical process giving rise to aggregation/polymerization thus generating structural alterations which may impact on the biological activity and potentially enhance immunogenicity.

[0010] Formulation development generally refers to a process in which an active pharmaceutical ingredient (API) is characterized to a sufficient extent that it can be converted to a pharmaceutically acceptable drug substance. Biophysical characterization of drug substances must be performed to confirm that the correctly folded and biologically active structure is present. Several spectroscopic techniques (Fluorescence, CD, DSC, DLS, etc.) can be used to examine the tertiary structure of proteins in solution and to assess the stability and effect of different formulations conditions on protein structure. (Volkin, D.B. et al. "Preformulation studies as an essential guide to formulation development and manufacture of protein pharmaceutical." *Development and manufacture of protein pharmaceuticals*. Edited by Steve L. Nail and Michael J. Akers, Kluwer Academic 2002 Chapter 1, page 1-39; Cheng, W. et al. "Comparison of High-Throughput Biophysical Methods to Identify Stabilizing Excipients for a Model IgG2 Monoclonal Antibody: Conformational Stability and Kinetic Aggregation Measurements" *Journal of Pharmaceutical Sciences*, Vol. 101, No 5, page 1701-1720, 2012). In addition, often the pharmaceutical industry confirms the structural integrity of the API by performing a biological assay for the release of the drug substance. Taken together they confirm that the preferred formulation has not inadvertently been modified by the formulation. Plasminogen is a protein that is mainly used in the preparation of plasmin for thrombolysis indications, such as coronary thrombosis, clearance of thrombotic occlusions in artificial devices and hemodialysis grafts, and for reperfusion injury to tissue, treating ischemia, infraction, brain edema, or for improving the microcirculation (WO 95/12407; US 6,969,515; EP 0,631,786).

[0011] The administration of plasminogen has also been found useful in many therapeutic indications such as thrombolysis indications, such as coronary thrombosis, treating reperfusion injury to tissue, treating ischemia, infarction, brain edema, or for improving the microcirculation, wound healing, healing of a tympanic membrane perforation, healing of periodontal wound, infectious disease, oral health, diabetic ulcer, plasminogen-deficient subjects, and modulation of complement pathway (WO 95/12407; EP 0,631,786 US 8,637,010; US 8,679,482; US 8,318,661).

[0012] There are several challenges encountered when formulating plasminogen. Some problems result from the plasmin contamination of the plasminogen formulation, which degrades plasminogen. Various approaches have been used for avoiding degradation of plasminogen including the addition of aprotinin, lysine, phenylmethanesulphonyl fluoride, soybean trypsin inhibitor or serine protease inhibitor (US 4,177,262; US 4,361,653, US 5,304,383).

[0013] Other difficulties are represented by the turbidity or the presence of filamentous substances when plasminogen is solubilised in an aqueous solvent. To overcome this drawback, it has been proposed to combine plasminogen for example with a nonionic surfactant or with a mixture of sucrose, amino acid and albumin (WO 94/15631).

[0014] US 4,297,344 discloses a plasminogen composition comprising an amino acid, in particular glycine, in combination with a carbohydrate, in particular saccharose/sucrose.

[0015] There is no known commercial formulation of Glu-Pg for human therapeutic use. The only formulations of Glu-Pg that are known are for research only.

[0016] There is a need for the development of a pharmaceutical composition of plasminogen.

BRIEF SUMMARY OF THE INVENTION

[0017] The scope of the invention is defined by the claims. Any references in the description to methods of treatment refer to the products of the present invention for use in a method for treatment.

[0018] The present invention relates to a pharmaceutical composition as defined in claim 1.

BRIEF DESCRIPTION OF THE FIGURES

[0019]

Figure 1A is a schematic of the primary structure of plasminogen showing its kringle domains, plasmin cleavage site and streptokinase binding site.

Figure 1B shows the amino acid sequence of human plasminogen precursor. The sequence of the mature form is in bold, and the two lysine residues between which there is a cleavage to generate Lys-Pg are underlined.

Figure 2 is a picture of a vial containing the placebo (without Pg) of Formulation 1 after lyophilisation.

Figure 3 is a picture a vial containing Formulation 1 (described in Table 1A) after lyophilisation.

Figure 4 is a picture a vial containing the placebo (without Pg) of Formulation 2 after lyophilisation.

Figure 5 is a picture a vial containing Formulation 2 (described in Table 1A) after lyophilisation.

Figure 6 is a picture a vial containing the placebo (without Pg) of Formulation 3 after lyophilisation.

Figure 7 is a picture a vial containing Formulation 3 (described in Table 1A) after lyophilisation.

Figure 8 is a picture a vial containing the placebo (without Pg) of Formulation 4 after lyophilisation.

Figure 9 is a picture a vial containing Formulation 4 (described in Table 1A) after lyophilisation.

Figure 10 is a picture a vial containing the placebo (without Pg) of Formulation 5 after lyophilisation.

Figure 11 is a picture a vial containing Formulation 5 (described in Table 1A) after lyophilisation.

Figure 12 a picture of vials of lyophilized composition no. F0498 which contains plasminogen 20 mg/ml in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, and 28.5 mM (0.5%) Arginine.

Figure 13 a picture of vials of lyophilized composition no. F0449 which contains plasminogen 10 mg/ml in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, and 54 mM (1%) Mannitol.

Figure 14 a picture of vials of lyophilized composition no. F0459 which contains plasminogen 20 mg/ml in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, and 54 mM (1%) Mannitol.

Figure 15 is a picture of vials containing 5 mg/ml plasminogen in water (Sample 0), in 10 mM sodium citrate buffer pH 6.5 (Sample 1), 10 mM sodium phosphate buffer pH 7.2 (Sample 2) and 10 mM Tris-HCl buffer Ph 8.0 (Sample 3) beside turbidity standards of 0.02, 20, 100, 800 NTU respectively.

Figure 16 is a picture of the same vials shown in Figure 12, with the exception that 35 mM NaCl and 28.5 mM (0.5%) Arg have been added to Samples 1, 2 and 3.

Figures 17, 18 and 19 are graphs showing the turbidity over a period of 4 hours for compositions containing 10 mg plasminogen, 35 mM NaCl with 0.5% glycine, arginine, alanine and without amino acid, at pH 6.5 in sodium citrate buffer (Figure 17), pH 7.2 in sodium phosphate buffer (Figure 18), and at pH 8.0 in Tris buffer (Figure 19).

Figures 20, 21 and 22 are graphs showing the turbidity over a period of 4 hours for compositions containing 10 mg plasminogen, 35 mM NaCl, 0.5% mannitol with 0.5% glycine, arginine, alanine and without amino acid, at pH 6.5 in sodium citrate buffer (Figure 20), pH 7.2 in sodium phosphate buffer (Figure 21), and at pH 8.0 in Tris buffer (Figure 22).

DETAILED DESCRIPTION OF THE INVENTION

[0020] The pharmaceutical composition according to the invention has a pH of about 5.0 to about 8.0; more preferably of about 6.0 to about 8.0; and further preferably of about 6.5 to about 7.5.

[0021] The present invention concerns a pharmaceutical composition with an increased robustness. The present invention concerns a pharmaceutical composition that has count of particles of 10 μm or greater that is lower than 6000 particles per 100 mL of composition, or lower than 5000 particles per 100 mL of composition, or lower than 4000 particles per 100 mL of composition, or lower than 3000 particles per 100 mL of composition, or lower than 2000 particles per 100 mL of composition or lower than 1000 particles per 100 mL of composition. In an embodiment of the present invention, the count of particles remains low after lyophilisation and reconstitution. In an embodiment of the present invention, repeated preparations of the same composition provide no variation of the count of particles.

[0022] Plasminogen is a pro-enzyme is converted to the active enzyme, plasmin, by tissue-type plasminogen activator (t-PA) or by urokinase plasminogen activator (u-PA). Plasmin then degrades fibrin and converts latent matrix metalloproteinases (pro-MMPs) into active MMPs, which in turn further degrade extracellular matrix (ECM) as part of the tissue healing/remodeling process. The term "biologically active variant" as used herein refers to a mutated plasminogen polypeptide that retains the biological activity of native plasminogen, i.e. the ability to be converted to a plasmin polypeptide (by t-PA and or u-PA) that is able to degrade fibrin and converts latent matrix metalloproteinases (pro-MMPs) into active MMPs. The biologically active variants may exhibit a biological activity (e.g., enzymatic activity of the resulting plasmin) that is lower, higher or similar to that of a native plasminogen polypeptide. In the embodiments according to the invention, the variant has at least 90, or 95% amino acid sequence identity with a native plasminogen polypeptide. Biologically active plasminogen variants are described, for example, in WO2012/093132, WO2013/024074 and in Wang et al. (1995, Protein Science 4, 1758-1767), and included the truncated variants of plasminogen commonly referred to as "midiplasminogen", "miniplasminogen", "microplasminogen" and

"delta-plasminogen" that lack one or more kringle domains and/or parts thereof. In an embodiment, the plasminogen is human plasminogen. In a further embodiment, the composition comprises native human plasminogen. The plasminogen may be obtained from several sources. It may be obtained by recombinant synthesis, or extracted/purified from blood, plasma or a blood-derived solution. Plasminogen can be extracted from blood or plasma by Cohn fractionation or by precipitation. Plasminogen can be purified from plasma or blood-derived solution by a binding affinity chromatography, such as the method described in WO 2006/120423, or produced recombinantly.

[0023] The plasminogen or biologically active variant thereof is present in a concentration of about 0.01 mg/ml to about 80 mg/ml; preferably about 1 mg/ml to about 60 mg/ml; preferably about 5 mg/ml to about 60 mg/ml; preferably about 5 mg/ml to about 40 mg/ml; preferably of about 2 mg/ml to about 30 mg/ml, more preferably of about 2 mg/ml to about 20 mg/ml, and further preferably of about 80, 70, 60, 50, 40, 30, 25, 20, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.5, 0.1, 0.05 or 0.01 mg/ml.

[0024] In an embodiment of the present invention, the plasminogen or biologically active variant thereof contained in the pharmaceutical composition has a purity of more than about 80%, or more than about 90%, or more than about 95%, or more than about 98%. The term "about" refers a variation of value by more or less 10%.

[0025] The pH of the present composition is from about 5.0 to about 8.0; from about 6.0 to about 8.0; or from about 6.5 to about 7.5. In embodiments, the pH of the present composition is about 5.0, 5.5, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5 or 8.0. To maintain the composition at such a pH, the present composition preferably comprises a buffer. Many buffers can be used within the scope of the present invention, including the following buffers depending on the desired pH (only compositions having a pH from about 5.0 to 8.0 are according to the claims):

Buffer	Desired pH
Citrate/phosphate	3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 to 7.0, 7.5, 8.0, and any pH from 2.0 and 8.0
Citrate	3.0 to 6.5
Acetate	any pH from 3.6 to 5.6, for example 4.0, 4.5, 5.0, or 5.5
Sodium acetate	3.6 to 5.6
Formate	3.0 to 4.5
Sodium formate	any pH from 5.0 and 6.0, for example 5.0, 5.5, or 6.0
Malate	4.0 to 6.0
Phosphate	any pH from 6.5 and 8.0, for example 6.5, 7.0, 7.5, or 8.0
PBS, TBS, TNT, PBT	7.0 to 7.5
Sorensen's	5.8 to 8.0
Succinate	any pH from 5.5 to 6.5, for example 6.0

Buffer	Desired pH
Carbonate	any pH from 6.0 to 8.0, for example 6.5
Histidine	any pH from 5.0 and 7.0, for example 5.0, 5.5, 6.0, or 6.5 to 7.0
Borate	9 to 10
Glycine-NaOH	8.6 to 10
Succinate	5.5 to 6.5
Maleate	5.5 to 7.2
Tris	7.2 to 9.0
BIS-Tris	any pH from 5.8 and 7.2
PIPES	any pH from 6.1 and 7.5
MOPS	any pH from 6.2 and 7.6
HEPES	any pH from 6.8 and 8.2
MES	any pH from 5.5 and 6.7
ACES	any pH from 6.1 and 7.5

[0026] In an embodiment, the buffer comprises a combination of two buffers; such as Citrate/Phosphate, Citrate/Histidine, Acetate/Histidine or Succinate/Histidine. In an embodiment of the present invention, the buffer does not comprise a citrate buffer. In another embodiment, the buffer is citrate, phosphate ($K_2HPO_4/NaHPO_4$), histidine, or succinate. In another embodiment, the buffer is phosphate ($K_2HPO_4/NaHPO_4$), histidine, or succinate.

[0027] In an embodiment, the concentration of the buffer is from about 10 mM to about 50 mM, or from about 10 mM to about 30 mM, for example about 2 mM or about 10 mM.

[0028] As used herein, a "tonicity modifier" refers to a compound which is used to adjust the tonicity of the pharmaceutical composition. Embodiments according to the claims have an osmolality between about 180 mOsm and 350 mOsm. In an embodiment, the tonicity modifier is present in an amount that renders the composition isotonic. In another embodiment, the tonicity modifier is present in an amount that renders the composition hypertonic or hypotonic. "Isotonic" is meant that the composition of the present invention has essentially the same osmolality than the osmolality of human blood. Isotonic compositions generally have an osmolality from about 250 to about 330 mOsm. Osmolarity can be measured using a vapor pressure or ice-freezing type osmometer, for example. The tonicity modifier is sodium chloride. In an embodiment, the concentration of the tonicity modifier concentration is about about 34 mM, or about 35 mM, or about 68 mM, or about 75 mM. In an embodiment, the concentration of the tonicity modifier is adjusted so that the resulting osmolality of the composition falls within the desired range.

[0029] In an embodiment, the composition further comprises a bulking agent. As used herein, a "bulking agent" refers to a compound/agent which increases the bulk mass of a composition. Examples of bulking agents include, without limitation, glutamic acid, alanine, mannitol, hydroethyl starch, dextran, PVP (poly vinyl pyrrolidone), mannitol, sorbitol, oligosaccharide-derived sugar alcohol, maltitol, lactitol, maltotriitol, xylitol, polyethylene glycol, sucrose, glucose, maltose, xorbitol, or any combination thereof. These agents can also serve as tonicity modifiers. In embodiments, the concentration of bulking agent is from about 50 mM to about 300 mM; or from about 100 mM to about 200 mM, or about 150 mM, or from about 50 mM to about 150 mM, or about 108 mM, or about 54 mM.

[0030] In an embodiment, the tonicity modifier and the bulking agent are in concentrations such that the tonicity modifier/bulking agent ratio is about 1.0 to about 15.0 (wt/wt); from about 1.0 to about 10.0, about 3.0 to about 10.0, about 5.0 to about 10.0, for example about 5.0, about 8.0, or about 10.0. In an embodiment, the concentration of bulking agent is adjusted based on the concentration of the tonicity modifier so that the tonicity modifier/bulking agent ratio is in the desired range.

[0031] In embodiments, the composition further comprises one or more additional pharmaceutically acceptable ingredients such as carriers, excipients, diluents, stabilizers, buffers and the like.

[0032] In an embodiment, the pharmaceutical composition further comprises a sugar, for example a reducing and/or a non-reducing sugar. As used herein, a "reducing sugar" is one which contains a hemiacetal group that can reduce metal ions or react covalently with lysine and other amino groups in proteins and a "non-reducing sugar" is one which does not have these properties of a reducing sugar. In an embodiment, the reducing sugar is fructose, mannose, maltose, lactose, arabinose, xylose, ribose, rhamnose, galactose, glucose or any combination thereof. In an embodiment, the non-reducing sugar is sucrose, trehalose, sorbose, melezitose, raffinose or any combination thereof. In an embodiment, the pharmaceutical composition of the present invention is lyophilized in the presence of a reducing sugar. In an embodiment, the pharmaceutical composition of the present invention is lyophilized in the presence of a non-reducing sugar.

[0033] The pharmaceutical composition of the present invention has an osmolality of about 180 mOsm to about 350 mOsm; or of about 250 mOsm to about 350 mOsm, or of about 200 mOsm to about 300 mOsm.

[0034] As used herein, a "stabilising agent" refers to an agent that stabilises plasminogen in solution. The stabilising agent is an amino acid which is arginine or glycine. In a preferred embodiment, said amino acid can be in the form of an amino acid base such as arginine hydrochloride hydrochloride. The concentration of amino acid is from about 10 mM to about 100 mM, or from about 25 mM to about 75 mM, or about 10 mM, or about 25 mM, or about 50 mM, or about 75 mM, or about 100 mM.

[0035] In another embodiment, the pharmaceutical composition of the present invention further comprises an antioxidant. In an embodiment, the antioxidant is methionine, oxidized glutathione (Glu-Cys-Gly)₂ (613 kDa), ascorbic acid, N-acetyl-L-cysteine/homocysteine, glutathione, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox[®]), lipoic acid, sodium thiosulphate, platinum, glycine-glycine-histidine (tripeptide), butylated hydroxytoluene (BHT), or any combination thereof. In embodiments, the concentration of the antioxidant is from about 10 mM to about 200 mM, e.g., about 10 mM, about 50 mM, about 100 mM, about 150 mM, or about 200 mM.

[0036] In another embodiment, the pharmaceutical composition of the present invention further comprises a surfactant. As used herein, a "surfactant" refers to a compound/agent that reduces interfacial tension between a liquid and a solid when dissolved in solution. In an embodiment, the surfactant is Polysorbate[®] 80, Polysorbate[®] 20, Pluronic[®] F-68, or Brij[®] 35; poloxamers (e.g. poloxamer 188 or Pluronic[®] F-68); Triton[®]; sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (e.g. lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUAT[™] series (Mona Industries, Inc., Paterson, N.J.), polyethyl glycol, polypropyl glycol, or copolymers of ethylene and propylene glycol, or any combination thereof. In an embodiment, the concentration of surfactant is adjusted to reduce aggregation of the plasminogen, biologically active variant or fragment thereof and/or minimizes the formation of particulates in the composition and/or reduces adsorption. In an embodiment, the surfactant is present in the composition in a concentration of about 0.001-1% (w/v), about 0.002% to about 0.02% (w/v), or about 0.002% to about 0.006% (w/v). In some embodiments, the composition comprises a surfactant which is a poloxamer. In some embodiments, the composition comprises Pluronic[®] F68. In particular embodiments, the composition comprises from about 0.01% (w/v) to about 1% (w/v) of Pluronic[®] F68, for example about 0.1% (w/v) of Pluronic[®] F68. In an embodiment, the composition is substantially free or free of nonionic surfactant, e.g., the composition comprises less than about 0.02%, 0.01%, 0.006%, or 0.004% (w/v) of nonionic surfactant.

[0037] In another embodiment, the pharmaceutical composition of the present invention further comprises a polymeric stabilizer. In an embodiment, the polymeric stabilizer is Heparin (6 to 30 kDa); Polyaminoacid (2 to 100 kDa) such as Poly (Glu), Poly (Asp), and Poly (Glu, Phe); Carboxymethyl cellulose (10-800cps); Cyclodextrin; Dextran Sulphate, or any combination thereof. In embodiments, the concentration of the polymeric stabilizer is from about 0.001% to about 0.50%, or from about 0.001% to about 0.25%.

[0038] In another embodiment, the pharmaceutical composition of the present invention further comprises a polyethylene glycol, e.g., PEG 200, PEG 400, PEG 1000, PEG, 4000, PEG,

8000, PEG 10 000, or any combination thereof. In an embodiment, the concentration of the polyethylene glycol is from about 0.5% to about 10% (w/w).

[0039] In an embodiment, the pharmaceutical composition of the present invention further comprises a preservative. As used herein, a "preservative" refers to a compound/agent which can be added to the pharmaceutical composition to essentially reduce bacterial activity therein, thus facilitating the production of a multi-use pharmaceutical composition, for example. Examples of preservatives include m-cresol, benzyl alcohol, methanol, ethanol, iso-propanol, butyl paraben, ethyl paraben, methyl paraben, phenol, glycerol, xylitol, resorcinol, catechol, 2,6-dimethylcyclohexanol, 2-methyl-2,4-pentadiol, dextran, polyvinylpyrrolidone, 2-chlorophenol, benzethonium chloride, merthiolate (thimersosal), benzoic acid (propyl paraben) MW 180.2, benzoic acid MW 122.12, benzalkonium chloride, chlorobutanol, sodium benzoate, sodium propionate, or cetylpyridinium chloride. The preservative is selected for compatibility with the buffer and other components of the pharmaceutical composition (i.e., the solution is clear). For example, when the buffer is sodium acetate or sodium phosphate, compatible preservatives include methanol, ethanol, iso-propanol, glycerol, resorcinol, 2-methyl-2,4-pentadiol, merthiolate (thimerosal), benzalkonium chloride, sodium benzoate, or cetylpyridinium chloride. The concentration of the preservative used in the present composition can be determined according to the judgment of those of skill in the art. In some embodiments, the concentration of the preservative is about 0.005 to about 10% (w/v), about 0.1 to about 1.0% (w/v), or about 0.3 to about 0.7% (w/v). In some embodiments, the concentration of the preservative is about 0.005, 0.1, 0.3, 0.5, 0.7, or 1.0% (w/v).

[0040] In another embodiment, the pharmaceutical composition of the present invention is free of preservative.

[0041] One or more additional pharmaceutically acceptable carriers, excipients or stabilizers, such as those described in Remington's Pharmaceutical Sciences 19th edition, Genarro, A. Ed. (1995) can be included in the present composition provided that they do not significantly adversely affect the desired characteristics of the pharmaceutical composition of the invention. Additional constituent elements of the composition of the present invention can include water, e.g., water for injection, vegetable oil, a thickening agent such as methylcellulose, an anti-adsorbant, a wetting agent, antioxidants including ascorbic acid and methionine, chelating agents such as EDTA, metal complexes (e.g. Zn-protein complexes), biodegradable polymers such as polyesters, and/or salt-forming counter-ions such as sodium etc. Acceptable carriers, excipients or stabilizers are present in an amount such that they are nontoxic to subjects at the dosages and concentrations employed.

[0042] In an embodiment, the pharmaceutical composition of the present invention has a total weight of solid of from about 3.5% to about 8% when lyophilized, preferably from about 3.5% to about 6%, and more preferably of from about 3.8% to about 5.5%. Increasing the concentration of components in the pharmaceutical composition of the invention results in increasing the total % of solid in the lyophilized composition and improving the cake appearance of the lyophilised composition.

[0043] In an embodiment, plasminogen or a variant thereof is composed various types of plasminogen and/or variants. In the embodiments according to the invention, the content of Glu-plasminogen represents more than 80% of the total content of plasminogen or variants thereof, or more than 90%.

[0044] An embodiment of the present invention is a pharmaceutical composition comprising plasminogen or a variant thereof, wherein the total amount of protein other than the plasminogen or variant thereof in the composition is less than about 10%, less than about 5%, or less than about 2%. An embodiment of the present invention is a pharmaceutical composition comprising plasminogen or a variant thereof, wherein the composition does not comprise or is substantially free of, an additional protein (i.e. in addition to the plasminogen or variant thereof). In an embodiment of the present invention, the composition does not comprise or is substantially free of albumin. In an embodiment of the present invention, the composition does not comprise or is substantially free of aprotinin. In an embodiment of the present invention, the composition does not comprise or is substantially free of a trypsin inhibitor. In an embodiment of the present invention, the composition does not comprise or is substantially free of a serine protease inhibitor. In an embodiment of the present invention, the composition does not comprise or is substantially free of plasmin. In an embodiment of the present invention, the composition is substantially free or free of a surfactant, i.e. the concentration of surfactant is less than 0.01 mM.

[0045] In an embodiment, the pharmaceutical composition comprising plasminogen of the present invention is stable at room temperature for at least 2 hours, 24 hours, a week or a month.

[0046] In an embodiment, the pharmaceutical composition comprising plasminogen of the present invention is stable at temperature below 0°C for at least 3 months, for at least 6 months, for at least 12 months or for at least 24 months. Said temperature below 0°C is about -20°C, about -30°C, about -60°C, about -70°C or about -80°C.

[0047] In embodiments, the pharmaceutical composition comprising plasminogen is a liquid composition, a liquid composition suitable for lyophilisation, a liquid composition suitable for freezing, a lyophilised composition, a frozen composition or a reconstituted composition.

[0048] The term "pharmaceutical composition" designates a composition for pharmaceutical, medical or therapeutic purposes.

[0049] Representative examples of pharmaceutical compositions of plasminogen (Pg) are represented in Table 1A. In Table 1A, only the compositions comprising NaCl in a concentration from about 10 mM to about 10 mM are according to the claims.

Table 1A

No.	Pg	Buffer	Bulking agent	Stabilising agent	Tonicity modifier	pH
1	5 mg/ml	10 mM Citrate	73 mM Sucrose	67 mM Glycine	68.4 mM NaCl;	6.5
2	5 mg/ml	10 mM Citrate	73 mM Sucrose	33.5 mM Glycine	34.22 mM NaCl	6.5
3	5 mg/ml	10 mM Citrate	117 mM Sucrose	67 mM Glycine	68.4 mM NaCl;	6.5
4	5 mg/ml	10 mM Citrate	117 mM Sucrose	67 mM Glycine	34.22 mM NaCl	6.5
5	5 mg/ml	10 mM Citrate	73 mM Sucrose	67 mM Glycine	34.22 mM NaCl	6.5
6	5 mg/ml	10 mM Citrate	73 mM Sucrose	67 mM Glycine	75.3 mM NaCl	6.5
7	5 mg/ml	10 mM Citrate	79 mM Sucrose	40 mM Glycine	75 mM NaCl	6.5
8	5 mg/ml	10 mM Citrate	77 mM Sucrose	47 mM Glycine	75 mM NaCl	6.5
9	5 mg/ml	10 mM Citrate	75 mM Sucrose	54 mM Glycine	75 mM NaCl	6.5
10	5 mg/ml	10 mM Citrate	50-150 mM Sucrose	25 to 75 mM Glycine	35-150 mM NaCl	6.5
11	5 mg/ml	10 mM Citrate	50-150 mM Mannitol	25 to 75 mM Glycine	35-150 mM NaCl	6.5
12	5 mg/ml	10 mM Citrate	50-150 mM Sorbitol	25 to 75 mM Glycine	35-150 mM NaCl	6.5
13	5 mg/ml	10 mM Citrate	50-150 mM Sucrose	25 to 75 mM Arginine	35-150 mM NaCl	6.5
14	5 mg/ml	10 mM Citrate	50-150 mM Mannitol	25 to 75 mM Arginine	35-150 mM NaCl	6.5
15	5 mg/ml	10 mM Citrate	50-150 mM Sorbitol	25 to 75 mM Arginine	35-150 mM NaCl	6.5
16	5 mg/ml	10 mM Phosphate	50-150 mM Sucrose	25 to 75 mM Glycine	35-150 mM NaCl	7.2
17	5 mg/ml	10 mM Phosphate	50-150 mM Mannitol	25 to 75 mM Glycine	35-150 mM NaCl	7.2
18	5 mg/ml	10 mM Phosphate	50-150 mM Sorbitol	25 to 75 mM Glycine	35-150 mM NaCl	7.2
19	5 mg/ml	10 mM Phosphate	50-150 mM Sucrose	25 to 75 mM Arginine	35-150 mM NaCl	7.2
20	5 mg/ml	10 mM Phosphate	50-150 mM Mannitol	25 to 75 mM Arginine	35-150 mM NaCl	7.2

No.	Pg	Buffer	Bulking agent	Stabilising agent	Tonicity modifier	pH
21	5 mg/ml	10 mM Phosphate	50-150 mM Sorbitol	25 to 75 mM Arginine	35-150 mM NaCl	7.2

[0050] Other representative examples of pharmaceutical compositions are represented in Table 1B below for compositions of about 0.01 mg/ml to about 80 mg/ml and preferably from 5 to 60 mg/ml of plasminogen or variant thereof and having a pH from 6.5 to 8.0. In all these examples, the content of plasminogen is composed of more than 80% Glu-plasminogen, and the total content of plasminogen or variants is from 5 mg/mL to 60 mg/mL. In table 1B, compositions 1, 12, 23-30 are not according to the claims.

Table 1B

No.	Bulking agent	Stabilising agent	Tonicity modifier
1	-	-	35 mM NaCl
2	-	25 mM Arginine HCl	35 mM NaCl
3	-	50 mM Arginine HCl	35 mM NaCl
4	50 mM Mannitol	25 mM Arginine HCl	35 mM NaCl
5	50 mM Mannitol	50 mM Arginine HCl	35 mM NaCl
6	100 mM Mannitol	25 mM Arginine HCl	35 mM NaCl
7	100 mM Mannitol	50 mM Arginine HCl	35 mM NaCl
8	50 mM Sorbitol	25 mM Arginine HCl	35 mM NaCl
9	50 mM Sorbitol	50 mM Arginine HCl	35 mM NaCl
10	100 mM Sorbitol	25 mM Arginine HCl	35 mM NaCl
11	100 mM Sorbitol	50 mM Arginine HCl	35 mM NaCl
12	-	-	50 mM NaCl
13	-	25 mM Arginine HCl	50 mM NaCl
14	-	50 mM Arginine HCl	50 mM NaCl
15	50 mM Mannitol	25 mM Arginine HCl	50 mM NaCl
16	50 mM Mannitol	50 mM Arginine HCl	50 mM NaCl
17	100 mM Mannitol	25 mM Arginine HCl	50 mM NaCl
18	100 mM Mannitol	50 mM Arginine HCl	50 mM NaCl
19	50 mM Sorbitol	25 mM Arginine HCl	50 mM NaCl
20	50 mM Sorbitol	50 mM Arginine HCl	50 mM NaCl
21	100 mM Sorbitol	25 mM Arginine HCl	50 mM NaCl
22	100 mM Sorbitol	50 mM Arginine HCl	50 mM NaCl
23	-	25 mM Arginine HCl	-
24	-	50 mM Arginine HCl	-

Table 1B			
No.	Bulking agent	Stabilising agent	Tonicity modifier
25	-	75 mM Arginine HCl	-
26	-	100 mM Arginine HCl	-
27	-	25 mM Arginine HCl	-
28	-	50 mM Arginine HCl	-
29	-	75 mM Arginine HCl	-
30	-	100 mM Arginine HCl	-

[0051] In an embodiment, the pharmaceutical composition comprising plasminogen of the present invention is suitable for intravenous, subcutaneous, topical, intradermal, ophthalmic and/or intramuscular administration.

[0052] According to an embodiment, the pharmaceutical composition of the present invention is for administration in a human subject. Preferably, the pharmaceutical composition of the present invention is for intravenous, subcutaneous, topical, intramuscular or ophthalmic administration. In an embodiment, the composition of the present invention is in the form of a liquid, gel, cream, or ointment.

[0053] The plasminogen that is formulated in the composition of the present invention may be obtained from several sources. It may be obtained by recombinant synthesis, or extracted/purified from blood, plasma or a blood-derived solution. Plasminogen can be extracted from blood or plasma by Cohn fractionation or by precipitation. Plasminogen can be purified from plasma or blood-derived solution by a binding affinity chromatography, such as the method described in WO 2006/120423.

[0054] As used herein, the term "about" intends to cover + or - 10% of the corresponding value.

[0055] The term "subject" includes living organisms which can benefit from an administration of plasminogen, a variant thereof or a fragment thereof. The term "subject" includes animals such as mammals or birds. Preferably, the subject is a mammal. More preferably, the subject is a human. Most preferably, the subject is a human patient in need of treatment.

[0056] As used herein, "preventing" or "prevention" is intended to refer to at least the reduction of likelihood of the risk of (or susceptibility to) acquiring a disease or disorder (i.e., causing at least one of the clinical symptoms of the disease not to develop in a patient that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease). Biological and physiological parameters for identifying such patients are provided herein and are also well known by physicians.

[0057] The terms "treatment" or "treating" of a subject includes the application or administration of a composition of the invention to a subject (or application or administration of the composition of the invention to a tissue or an organ of a subject) with the purpose of delaying, stabilizing, curing, healing, alleviating, relieving, altering, remedying, less worsening, ameliorating, improving, or affecting the disease or condition, the symptom of the disease or condition, or the risk of (or susceptibility to) the disease or condition. The term "treating" refers to any indication of success in the treatment or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement; remission; lessening of the rate of worsening; lessening severity of the disease; stabilization, diminishing of symptoms or making the injury, pathology or condition more tolerable to the subject; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; or improving a subject's physical or mental well-being. In some embodiments, the term "treating" can include increasing a subject's life expectancy and/or delay before additional treatments are required.

[0058] As used herein, the term "therapeutically effective amount" means the amount of compound that, when administered to a subject for treating or preventing a particular disorder, disease or condition, is sufficient to effect such treatment or prevention of that disorder, disease or condition. As used herein, the term "therapeutically effective amount" further means the amount of plasminogen, its variant or its fragment that is effective to heal a wound, heal a tympanic membrane perforation, heal a periodontal wound, treat an infectious disease, increase or maintain oral health, treat posterior vitreous detachment (PVD), diabetic ulcer, and plasminogen-deficient subjects thrombolysis indications, such as coronary thrombosis, treating reperfusion injury to tissue, treating ischemia, infarction, brain edema, for improving the microcirculation, treat plasminogen-deficient subjects, and modulate of complement pathway in subjects. Dosages and therapeutically effective amounts may vary for example, depending upon a variety of factors including the activity of the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, and any drug combination, if applicable, the effect which the practitioner desires the compound to have upon the subject, and the particular disorder(s) the subject is suffering from. In addition, the therapeutically effective amount may depend on the subject's blood parameters (e.g., lipid profile, insulin levels, glycemia), the severity of the disease state, organ function, or underlying disease or complications. Such appropriate doses may be determined using any available assays including the assays described herein. When the pharmaceutical composition of the invention is to be administered to humans, a physician may for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. The dose to be administered will ultimately be at the discretion of the physician.

Kits

[0059] The compound(s) of the invention may be packaged as part of a kit, optionally including a container (e.g., packaging, a box, a vial, etc.). The kit may be commercially used according

to the methods described herein and may include instructions for use in a method of the invention.

[0060] The composition of the invention may or may not be administered to a patient at the same time than another active ingredient, before or after, and by the same route of administration or by another route of administration. Therefore, the present invention also encompass kits which, when used by the medical practitioner, can simplify the administration of appropriate amounts of the present composition alone or in combination with another active ingredient to a patient.

[0061] Kits of the invention can further comprise a pharmaceutically acceptable liquid for reconstitution of a lyophilised composition. For example, if an active ingredient is provided in a solid form that must be reconstituted for parenteral administration, the kit can comprise a sealed container of a suitable pharmaceutically acceptable liquid in which the active ingredient can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration.

[0062] In certain embodiments, the present composition is contained in a vial, bottle, tube, syringe or other container for single or multiple administrations. Such containers can be made of glass or a polymer material such as polypropylene, polyethylene, polyvinylchloride, or polyolefin, for example. In some embodiments, the containers can include a seal, or other closure system, such as a rubber stopper that can be penetrated by a needle in order to withdraw a single dose and then re-seal upon removal of the needle. All such containers for injectable liquids, lyophilized compositions, reconstituted lyophilized compositions or powders for reconstitution and injection known in the art are contemplated for use in the presently disclosed compositions and methods. In a particular embodiment, the container is a pen-type delivery apparatus comprising a single dose or multiple doses. Such a pen-type delivery apparatus can be permanent, e.g., a permanent pen that houses a disposable cartridge containing a single dose or multiple doses, or the entire apparatus can be disposable, e.g., a disposable pen that contains a single dose or multiple doses. In certain embodiments where the pen-type delivery apparatus comprises multiple doses, the dose can be pre-set, i.e., fixed. In other embodiments, the dose can be a flexible dose, i.e., dialed-in by the user. In some embodiments, the pen-type delivery apparatus comprises a luer-lock, luer-cone, or other needle fitting connector that facilitates attachment of a disposable needle. In other embodiments, the pen-type delivery apparatus comprises a staked, i.e., permanent needle. In another particular embodiment, the container is a syringe. In some embodiments, the syringe comprises a luer-lock, luer-cone, or other needle fitting connector that facilitates attachment of a disposable needle. In other embodiments, the syringe comprises a staked, i.e., permanent, needle. In some embodiments, the syringe is prefilled with a single dose or multiple doses.

Stability assays

[0063] "Stable" compositions include compositions in which the protein (plasminogen or variant

thereof) therein essentially retains its physical stability and/or chemical stability and/or biological activity upon storage. Various analytical techniques for measuring protein stability are available in the art and are reviewed in Lee, V., 1991, Peptide and Protein Drug Delivery, 247-301 (Marcel Dekker, Inc., New York, N.Y.) and Jones, A. 1993, Adv. Drug Delivery Rev. 10: 29-90, for example. Stability can be measured at a selected temperature for a selected time period. In an embodiment, the composition is stable at room temperature (about 25°C) or at 40°C, for at least 1, 2, 3, 4, 5 or 6 months and/or stable at about 2-8°C for at least 1, 2, 3, 4, 5 or 6 months. Furthermore, in certain embodiments, the composition is stable following freezing (e.g., -30°C.). In certain embodiments, the criteria for stability are as follows: (1) the composition remains clear by visual analysis; (2) the concentration, pH and osmolality of the composition has no more than about $\pm 10\%$ change; (3) no more than about 10%, no more than about 5%, or no more than about 1% of aggregate forms as measured by SEC-HPLC; and (4) no more than 10%, no more than about 5%, or no more than 3% of plasminogen breaks down as measured by known in the art and well established analytical methods.

[0064] Thermal Denaturation: The thermal denaturation experiments HTS formulation screening for Plasminogen or a variant thereof or a fragment thereof can performed using Optim 2™ from Pall; or other alternative biophysical technique(s) to monitor the unfolding of the protein (Standard PCR (with a heating block), Fluorimeter or CD spectrophotometer (with temperature control). As an example, the Optim 2 system simultaneously measure a range of protein stability-indicating parameters including unfolding transition temperature (T_m), aggregation onset temperature (Tagg) and rates of aggregation.

[0065] The effects of excipients on the thermostability: Narrow pH is determined at the optimum pH range with higher Melting Temperature (T_m) and Aggregation Onset Temperature (Tagg). If the higher T_m is obtained in the physiological range of 6.5-7.5; the next test(s) is to screen with at least 3 buffer system(s).

[0066] Additionally, IEF (isoelectric focusing) or cIEF (capillary isoelectric focusing) can be tested for evaluation the physical conformation of plasminogen in solution.

[0067] The percentage of stabilization based on plasminogen enzyme activity measurements, for each buffer/excipient and for a reference solution containing only plasminogen after 1-8 hours of incubation at 60°C. In another test, the stabilization may be evaluated at 37°C for 1-8 hours. The percentage of stabilization is calculated as:
Percent of Stabilization = $100 \times [S - T] / [T]$

[0068] Where S is the residual enzyme activity of the enzyme solution with the excipient to be tested after 10 days at 35°C; and T is the activity of the reference without any excipient stored in the same conditions.

[0069] Aggregation and Particle Measurement Count (PMC): The PMC is calculated by the Light Obscuration Particle Count Test. Analysts should use a suitable instrument that is based

on the principle of light blockage and that allows an automatic determination of particle size and number. The sensor selected must be appropriate for the intended particle size range and anticipated particle count. Standards particle size range are generally between 2 and 100 μm . Analysts should verify the performance of the apparatus using the USP Particle Count RS dispersed in particle-free water at an appropriate volume. Care must be taken to avoid agglomeration of particles during dispersion in the calibration process.

[0070] Methods: Product samples are tested in the manner that most suitably represents delivery (e.g., expelled syringe contents). For parenterals that have a sufficient volume (i.e., volume large enough to facilitate testing), testing of individual units is often diagnostic. For parenteral products that do not have a sufficient volume, carefully and thoroughly mix each unit, then combine the contents of a suitable number of units in a separate container to obtain the volume required for a single test (generally 0.2-5.0 mL). If a dilution is performed, ensure that the blank container has sufficient volume for product and diluents and that few, if any, particles are introduced in the process. Open each unit cautiously, remove the sealing closure, and avoid contamination of the contents before sampling, diluting, or, if necessary, pooling. When a product solvent is specified, e.g., for lyophilized solids or powders for parenteral use, the reconstitution or dilution must be performed with the appropriate amount of specified solvent. In this case, the solvent itself may be tested to ensure that it is not a significant source of particles. Subtraction of the solvent particle count from the total count is not allowed. Eliminating gas bubbles is a key step, especially for proteinaceous products that readily entrain gas. Two methods are recommended: either allowing the product fluid to stand under ambient pressure or applying a gentle (e.g., 75 Torr) vacuum. Other methods may be used when demonstrated to be suitable. Sonication should be avoided. Once the samples have been degassed, they must be remixed gently to suspend all particles by mixing the contents of the sample gently but thoroughly by an appropriate means, e.g., slow swirling of the container by hand. Inversion to mix the product fluid is not recommended at any time. Immediately after mixing, withdraw NLT four aliquots, each of a volume appropriate for the instrument's capacity (generally 0.2-5.0 mL). Count the number of particles over the selected size range, including particles equal to or greater than 10 and 25 μm . Disregard the result obtained for the first aliquot, and calculate the mean number of particles at each size range for the remaining aliquots of the preparation being tested. A tare volume also can be applied to control sampling that should be representative of the sensor dynamic range and needle volume..

[0071] Evaluation: Regulatory authorities like the FDA (Food and Drug Administration) and USP/EP/JP (United State Pharmacopoeia) Chapters (787, 788 and 789), EP European Pharmacopoeia (2.9.20 Particulate Contamination), and JP Japanese Pharmacopoeia JP (16: 6.06. 6.07 Foreign Insoluble Matter Test) requirements on Particulate Matter define the acceptable level of particles in liquid preparation for human use as follow. For parenteral products that are therapeutic protein injections for infusion or injection supplied in containers with a nominal content of less than or equal to 100 mL: the average number of particles present in the units tested should not exceed 6000 per container equal to or greater than 10 μm and should not exceed 600 per container equal to or greater than 25 μm . For therapeutic protein injections supplied in containers with a nominal content of more than 100 mL, and

parenteral infusion preparations or injections with a nominal content of more than 100 mL: the average number of particles present in the units tested should not exceed 25 per mL equal to or greater than 10 µm and should not exceed 3 per mL equal to or greater than 25 µm. Also, total particle load should not exceed 6000 per container equal to or greater than 10 µm and should not exceed 600 per container equal to or greater than 25 µm. Products that are used with a final filter during administration (in-line) are exempt from these requirements, providing that scientific data are available to justify the exemption. However, filtrates are expected to comply with the guideline. For products supplied or first reconstituted in <100 mL, and then diluted for infusion in a volume >100 mL, particle content should be assessed both before and after dilution and evaluated based on their final volume. Hence, USP/EP/JP Method are herein included in this document. Herein, the term "Particle" refers to "Particulate" and vice-versa; these terms can be used interchangeably.

[0072] Kinetic Study Measured by light scattering at 476 nm / 600 nm using the Optim-2™ or by Standard UV Spectrophotometer at UV at 350 nm: Accelerated conditions such as elevated temperature (65°C) and acidic solution pH (4.5) or basic solution pH (9.5) is selected to more rapidly screen for different stabilizing excipients. The components of the composition are evaluated to increase the Tagg as a function of time at a fixed temperature (55-60°C) for at least 1 to 4 hours. The aggregation kinetic behavior of the Plasminogen solution is measured at 55-60°C equipped with temperature control (onset Tm is determined) for up to 4h. The excipients are screened for their effect on inhibition of Plasminogen aggregation as measured by the light scattering at 267 nm, 467 nm or 350 nm (Cheng, W. et al. "Comparison of High-Throughput Biophysical Methods to Identify Stabilizing Excipients for a Model IgG2 Monoclonal Antibody: Conformational Stability and Kinetic Aggregation Measurements" Journal of Pharmaceutical Sciences, Vol. 101, No 5, page 1701-1720, 2012).

[0073] The effect of protein concentration of thermal stability: One of the advantages of Optim-2™ or Dual Scanning Fluorimeter (DSF with the Sypro Dye) is the ability to carry out measurements of Tm and Tagg in highly concentrated protein solutions. Whether investigating potential candidates for relative stability, or screening different formulation conditions for stabilization of proteins, it may be important to identify the properties of the protein solution at high concentrations where the number of characterization methods available is limited.

[0074] The effect of formulation composition on the final Osmolality: In the final pharmaceutical composition, the buffer, excipient and salt concentrations are not preferred to be very high due to the practical limitations of parental injection. Instead, more moderate concentrations such as more physiological are preferred in order to obtain an Osmolality in the range of 240 to 400 mOsm. Therefore, Osmolality is calculated to initially determine this range; however for IV injection a higher Osmolality concentrations can also be used during the development of the freeze drying process if after reconstitution Plasminogen or variant or fragment thereof is diluted by the solution for reconstitution.

[0075] It should be noted that the term "Formulation" and "Composition" may be used herein alternatively and are intended to designate the same.

[0076] The singular forms "a", "an" and "the" include corresponding plural references unless the context clearly dictates otherwise.

[0077] Unless otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, concentrations, properties, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about". At the very least, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the present specification and attached claims are approximations that may vary depending upon the properties sought to be obtained. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the embodiments are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contain certain errors resulting from variations in experiments, testing measurements, statistical analyses and such.

EXAMPLES

[0078] The following examples further illustrate the practice of this invention but are not intended to be limiting thereof.

[0079] The examples according to the claims are the ones comprising:

- Glu-plasminogen in a concentration from about 0.01 mg/ml to about 80 mg/ml;
- sodium chloride present in a concentration of between about 30 mM to about 100 mM;
- and
- arginine or glycine, in a concentration from about 10 mM to about 100 mM;

wherein the composition has a pH from about 5.0 to about 8.0; and

wherein the osmolality of the composition is between about 180 mOsm and about 350 mOsm.

Example 1: Stability of Plasminogen in a Frozen Formulation

[0080] Stability testing results for a pharmaceutical composition comprising 5 mg/ml of human plasminogen extracted from plasma in accordance with a binding affinity technique described in WO 2006/120423, 10 mM Citrate, 150 mM NaCl, at pH 6.5 (Lot: 2002.pc02_Pg140210.01) were stored at -20°C, -30°C and -80°C for up to 6 Months. The stability data indicated that plasminogen is stable under these frozen conditions. Plasminogen lot: 2002.pc02_Pg140210.01 was tested at -20°C, -30°C and -80°C. The level of aggregation was

investigated by SEC-HPLC, the level of purity by SDS-PAGE under reducing, and also under non-reducing conditions, the level of activity by BCS activity and the protein concentration by absorbance at 280 nm (ABS₂₈₀). The results of the stability data after 6 months demonstrated that all the stability indicating assays achieved the desired specifications.

Example 2: Osmolality analysis and Total Percentage of Solid analysis

[0081] The osmolality of formulations 1 to 6 and the total percentage of solid after lyophilisation for formulations 1-9 are reported in Table 2. The content of Formulations 1-9 is defined in Table 1A.

Table 2

Formulation	Plasminogen	Sucrose	Glycine	NaCl	Total % of Solid Cake	Osmolality
#	mg/mL	mM	mM	mM	%	mOsm
1	5	73	67	68	3.9	265
2	5	73	34	34	3.4	198
3	5	117	67	68	5.4	309
4	5	117	67	34	5.2	275
5	5	73	67	34	3.7	231
6	5	73	67	75	3.9	272
7	5	27	40	75	3.9	TBD
8	5	26.5	47	75	3.9	TBD
9	5	26	54	75	3.9	TBD

[0082] The osmolality is in the physiological range of 240-400 mOsm. The resulting osmolality of the tested compositions are advantageously falling within or very close to the physiological range of osmolality. The total % of solid cake (g of solid composition after lyophilisation per 100 ml of the liquid composition before lyophilization) is around 4% for all compositions and placebos, which is within the preferred range of 3.5 to 8%.

Example 3: Molecular weight of plasminogen

[0083] The molecular weight of the plasminogen purified and formulated in the pharmaceutical composition of the present invention has a molecular weight of 87,000 (MW), determined by calculation of the primary sequence of amino acids of the plasminogen and verified by mass spectrometry.

Example 4: Study of Appearance of the Cake after Lyophilisation

[0084] Formulations 1 to 5 have been lyophilised following the cycle process detailed in Table 5. The content of Formulations 1-5 is defined in Table 1A.

Table 5

Step	Shelf Temperature Set Point, [°C]	Ramp Time, [minutes]	Soak Time, [minutes]	Pressure Set Point, [mT]
Product Loading	5	-	60	Atmospheric
Freezing	-50	120	240	Atmospheric
Evacuation	-50	-	-	50
Primary	-22	90	3860	50
Secondary	35	120	270	50

[0085] Figures 2 to 11 are pictures of the vials containing 12.5 ml of Formulations 1 to 5 respectively, or their corresponding placebo (without plasminogen). Thus, the effect of the presence of plasminogen on the stability of the composition has been investigated. The physical inspection of the appearance of the cake that has been formed by the lyophilisation process. All cakes have maintained a uniform density, without any discoloration. Less than 10% of the tested vials for the placebo of Formulations 2, 3 and 5 have provided a melt back. Less than 10% of the tested vials for the placebo of Formulation 1 have collapsed. Less than 10% of the tested vials for the Formulations 1-5 and the placebo of Formulation 4 have shown a basal retraction. Overall the appearance of the cake is satisfying.

[0086] The content of the composition dictates collapse temperature. Each pure amorphous excipient has a characteristic Tg' and collapse temperature; the collapse temperature for the formulation is the mass averaged temperatures of all of the compositions in the amorphous phase. It is important to design a formulation with maximum collapse temperature, because the rate of drying is directly proportional to the sample temperature during lyophilization.

[0087] Finally, collapse temperature will be decreased if salts (NaCl) and excipients are not maximally crystallized. For example, glycine has a Tg' of -45°C, and its contribution to the amorphous phase can reduce collapse temperature to impractically low values.

Example 5: Effect of amino acid on plasminogen aggregation in solution

[0088] Plasminogen alone in water generates a number of plasminogen aggregates that is unacceptable by the FDA i.e. higher than 6000 particles of 10 µm or greater per 100 mL of composition (see Sample F0406 in Table 6). Various sucrose-based formulations have been developed to formulate plasminogen. Although most of these formulations succeed to meet the

threshold of 6000 particles of 10 µm or greater per 100 mL, formulations containing a lower particle content have been developed and the effect of amino acid to stabilise plasminogen is studied herein. These further formulations contain 10 mg of plasminogen and 35 mM NaCl, and three buffers/pH have been tested, i.e. 10 mM Sodium Citrate (pH 6.5), 10 mM Sodium Phosphate (pH 7.2) and 10 mM Tris HCL (pH 8.0). Have been compared the presence and absence of an amino acid selected from arginine, alanine and glycine at a concentration of 0.5%. The tested formulations are fresh, i.e. before lyophilisation. Table 6 reports the PMC counts for the particles of 10 µm or greater and of 25 µm or greater. The presence of amino acid have shown a stabilizing effect has contributed to reduce the aggregation level.

Table 6

Assigned Sample ID	Sample Description	Pre-Lyo PMC Count/mL	
		10 µm	25 µm
F0406	Pg Bulk in water at 10 mg/ml	1,039,061	20,181
Rx1	Pg (5 mg/mL), 67 mM glycine	5,793	155
Rx5	Pg (5 mg/mL), 12 mM citrate buffer, 73 mM sucrose, 68 mM NaCl, 67 mM glycine, pH 6.5	5,495	243
Rx6	Pg (5 mg/mL), 12 mM citrate buffer, 117 mM sucrose, 68 mM NaCl, pH 6.5	4,068	94
Rx7	Pg (5 mg/mL), 12 mM citrate buffer, 117 mM sucrose, 68 mM NaCl, pH 6.5	3,349	96
F0080	Pg (10 mg/mL), 10 mM Sodium Citrate, 35 mM NaCl, pH 6.5	147	0
F0081	Pg (10 mg/mL), 10 mM Sodium Citrate, 35 mM NaCl, 66 mM (0.5%) Glycine , pH 6.5	109	0
F0082	Pg (10 mg/mL), 10 mM Sodium Citrate, 35 mM NaCl, 28.5 mM (0.5%) Arginine , pH 6.5	122	1
F0083	Pg (10 mg/mL), 10 mM Sodium Citrate, 35 mM NaCl, 26 mM (0.5%) Alanine , pH 6.5	91	1
F0102	Pg (10 mg/mL), 10 mM Sodium Phosphate, 35 mM NaCl, pH 7.2	271	18
F0103	Pg (10 mg/mL), 10 mM Sodium Phosphate, 35 mM NaCl, 66 mM (0.5%) Glycine , pH 7.2	124	6
F0104	Pg (10 mg/mL), 10 mM Sodium Phosphate, 35 mM NaCl, 28.5 mM (0.5%) Arginine , pH 7.2	128	3
F0105	Pg (10 mg/mL), 10 mM Sodium Phosphate, 35 mM NaCl, 26 mM (0.5%) Alanine , pH 7.2	180	2
F0106	Pg (10 mg/mL), 10 mM Tris, 35 mM NaCl,	118	5

Assigned Sample ID	Sample Description	Pre-Lyo PMC Count/mL	
		10 µm	25 µm
	pH 8.0		
F0107	Pg (10 mg/mL), 10 mM Tris, 35 mM NaCl, 66 mM (0.5%) Glycine , pH 8.0	91	7
F0108	Pg (10 mg/mL), 10 mM Tris, 35 mM NaCl, 28.5 mM (0.5%) Arginine , pH 8.0	75	3
F0109	Pg (10 mg/mL), 10 mM Tris, 35 mM NaCl, 26 mM (0.5%) Alanine , pH 8.0	46	1

Example 6: Effect of mannitol combined with amino acid on plasminogen aggregation

[0089] The presence of the bulking agent, 0.5% mannitol, on the formulations containing 10 mg of plasminogen and 35 mM NaCl, with either 10 mM Sodium Citrate (pH 6.5), 10 mM Sodium Phosphate (pH 7.2) or 10 mM Tris HCL (pH 8.0), with and without amino acid (0.5% arginine hydrochloride, alanine or glycine) have been tested and reported in Table 7. Fresh formulations (before lyo) were tested in this study. Generally, the presence of mannitol has lowered the particle counts. Generally, the combination of mannitol and amino acid contributes to lower the particle counts, and especially with the combination of arginine with mannitol.

Table 7

Assigned Sample ID	Sample Description	Pre-Lyo PMC Count/mL	
		10 µm	25 µm
F0114	Pg (9 mg/mL), 10 mM Sodium Citrate, 35 mM NaCl, pH 6.5	3,610	61
F0115	Pg (9 mg/mL), 10 mM Sodium Citrate, 35 mM NaCl, 27 mM (0.5%) Mannitol, pH 6.5	805	8
F0116	Pg (9 mg/mL), 10 mM Sodium Citrate, 35 mM NaCl, 66 mM (0.5%) Glycine , 27 mM (0.5%) Mannitol, pH 6.5	962	13
F0117	Pg (9 mg/mL), 10 mM Sodium Citrate, 35 mM NaCl, 28.5 mM (0.5%) Arginine , 27 mM (0.5%) Mannitol, pH 6.5	520	11
F0118	Pg (9 mg/mL), 10 mM Sodium Citrate, 35 mM NaCl, 26 mM (0.5%) Alanine , 27 mM (0.5%) Mannitol, pH 6.5	990	1
F0119	Pg (9 mg/mL), 10 mM Sodium Phosphate, 35 mM NaCl, pH 7.2	133	6
F0120	Pg (9 mg/mL), 10 mM Sodium Phosphate, 35	107	3

Assigned Sample ID	Sample Description	Pre-Lyo PMC Count/mL	
		10 μ m	25 μ m
	mM NaCl, 27 mM (0.5%) Mannitol, pH 7.2		
F0121	Pg (9 mg/mL), 10 mM Sodium Phosphate, 35 mM NaCl, 66 mM (0.5%) Glycine , 27 mM (0.5%) Mannitol, pH 7.2	191	6
F0122	Pg (9 mg/mL), 10 mM Sodium Phosphate, 35 mM NaCl, 28.5 mM (0.5%) Arginine , 27 mM (0.5%) Mannitol, pH 7.2	95	2
F0123	Pg (9 mg/mL), 10 mM Sodium Phosphate, 35 mM NaCl, 26 mM (0.5%) Alanine , 27 mM (0.5%) Mannitol, pH 7.2	113	5
F0124	Pg (9 mg/mL), 10 mM Tris, 35 mM NaCl, pH 8.0	86	2
F0125	Pg (9 mg/mL), 10 mM Tris, 35 mM NaCl, 27 mM (0.5%) Mannitol, pH 8.0	578	10
F0126	Pg (9 mg/mL), 10 mM Tris, 35 mM NaCl, 66 mM (0.5%) Glycine , 27 mM (0.5%) Mannitol, pH 8.0	163	5
F0127	Pg (9 mg/mL), 10 mM Tris, 35 mM NaCl, 28.5 mM (0.5%) Arginine , 27 mM (0.5%) Mannitol, pH 8.0	13	3
F0128	Pg (9 mg/mL), 10 mM Tris, 35 mM NaCl, 26 mM (0.5%) Alanine , 27 mM (0.5%) Mannitol, pH 8.0	119	8

Example 7: Effect of preservative on plasminogen aggregation

[0090] The presence of preservative, such as 0.1% phenol, has been tested in the formulations containing 9 or 10 mg of plasminogen and 35 mM NaCl, with either 10 mM Sodium Citrate (pH 6.5), 10 mM Sodium Phosphate (pH 7.2) or 10 mM Tris HCL (pH 8.0), with 0.5% arginine hydrochloride or the combination of 0.5% arginine hydrochloride and 0.5% mannitol. Fresh formulations (before lyo) were tested in this study. The particle matter counts (PMC) have been reported in Table 8. Advantageously, the presence of phenol has not significantly affected the particle counts.

Table 8

Assigned Sample ID	Sample Description	Pre-Lyo PMC_Count/mL	
		10 μ m	25 μ m
F0114	Pg (9 mg/mL), 10 mM Sodium Citrate, 35 mM NaCl, pH 6.5	3,610	61
F0143	Pg (10 mg/mL), 10 mM Sodium Citrate, 35 mM NaCl, 28.5 mM (0.5%) Arginine, pH 6.5	609	8
F0144	Pg (10 mg/mL), 10 mM Sodium Citrate, 35 mM NaCl, 28.5 mM (0.5%) Arginine, 0.1% Phenol , pH 6.5	3,191	27
F0145	Pg (10 mg/mL), 10 mM Sodium Citrate, 35 mM NaCl, 28.5 mM (0.5%) Arginine, 27 mM (0.5%) Mannitol, 0.1% Phenol , pH 6.5	1,538	9
F0155	Pg (10 mg/mL), 10 mM Sodium Phosphate, 35 mM NaCl, 28.5 mM (0.5%) Arginine, pH 7.2	1,097	7
F0156	Pg (10 mg/mL), 10 mM Sodium Phosphate, 35 mM NaCl, 28.5 mM (0.5%) Arginine, 0.1% Phenol , pH 7.2	4,370	6
F0157	Pg (10 mg/mL), 10 mM Sodium Phosphate, 35 mM NaCl, 28.5 mM (0.5%) Arginine, 27 mM (0.5%) Mannitol, 0.1% Phenol , pH 7.2	747	9
F0158	Pg (10 mg/mL), 10 mM Tris, 35 mM NaCl, 28.5 mM (0.5%) Arginine, pH 8.0	169	4
F0159	Pg (10 mg/mL), 10 mM Tris, 35 mM NaCl, 28.5 mM (0.5%) Arginine, 0.1% Phenol , pH 8.0	168	3
F0160	Pg (10 mg/mL), 10 mM Tris, 35 mM NaCl, 28.5 mM (0.5%) Arginine, 27 mM (0.5%) Mannitol, 0.1% Phenol , pH 8.0	175	1

[0091] Several preservatives were compared. Table 9 reports comparison of 0.5% CMC, 0.1% phenol and 0.5% dextran within the formulations containing 10 mg of plasminogen and 35 mM NaCl, with either 10 mM Sodium Citrate (pH 6.5), 10 mM Sodium Phosphate (pH 7.2) or 10 mM Tris HCL (pH 8.0), and 0.5% arginine hydrochloride. Fresh formulations (before lyo) were tested in this study. The presence of dextran or phenol has not significantly affected the aggregation. However, CMC has significantly increased the aggregation. This is not surprising since CMC is known to increase the viscosity of a solution and is frequently used in the preparation of eye drop formulation.

Table 9

Sample ID	Sample Description	Pre-Lyo PMC Count/mL	
		10 µm	25 µm
F0119	Pg (9 mg/mL), 10 mM Sodium Phosphate, 35 mM NaCl, pH 7.2	133	6
F0569	Pg (10 mg/mL) Bulk in 10 mM Na Phosphate, 35 mM NaCl, 28.5 mM (0.5%) Arginine, pH 7.2, Filtered (F0199), F/T=1x	19	1
F0570	Pg (10 mg/mL), 10 mM Sodium Phosphate, 35 mM NaCl, 28.5 mM (0.5%) Arginine, 0.5% CMC , pH 7.2	7,783	1
F0571	Pg (10 mg/mL), 10 mM Sodium Phosphate, 35 mM NaCl, 28.5 mM (0.5%) Arginine, 0.1% Phenol , pH 7.2	25	1
F0572	Pg (10 mg/mL), 10 mM Sodium Phosphate, 35 mM NaCl, 28.5 mM (0.5%) Arginine, 0.5% Dextran , pH 7.2	37	1

Example 8: Effect of amino acid and its concentration on plasminogen aggregation

[0092] Arginine at 0.5% and 1% were tested in formulations of Plasminogen 10 mg/mL, 35 mM NaCl, 10 mM Sodium Phosphate (NaPi), pH 7.2, after lyophilisation and reconstitution. The particle counts (PMC) are reported in Table 10, which shows that both concentration of arginine are very good to maintain a low PMC value.

Table 10

Assign Sample ID	Sample Description	Reconstitution in dH2O (ml)	Volume (ml)	Pre-Lyo PMC Count/mL	
				10 µm	25 µm
F0211	Pg 10 mg/mL, 28 mM (0.5%) Arg , 35mM NaCl, 10 mM NaPi, pH 7.2	12.5	4	87	2
F0213	Pg 10 mg/mL, 57 mM (1%) Arg , 35 mM NaCl, 10 mM NaPi, pH 7.2	12.5	4	124	11

[0093] Arginine, glycine and alanine at 1% were compared in formulations of Plasminogen 10 mg/mL, 35 mM NaCl, 10 mM Sodium Phosphate (NaPi), pH 7.2, after lyophilisation and reconstitution. The particle counts (PMC) are reported in Table 11, which shows that arginine is better than alanine and glycine to maintain a low PMC value in the tested conditions, although

all these three amino acids provide acceptable PMC values i.e. below 6000 particles equal to or greater than 10 μm per 100 mL.

Table 11

Assign Sample ID	Sample Description	Reconstitution In dH ₂ O (ml)	Volume (ml)	Pre-Lyo PMC_Count/mL	
				10 μm	25 μm
F0215	Pg 10 mg/mL, 57 mM (1%) Arg , 35 mM NaCl, 10 mM NaPi, pH 7.2	12.5	4	178	6
F0217	Pg 10 mg/mL, 57 mM (1%) Gly, 35mM NaCl, 10 mM NaPi, pH 7.2	12.5	4	881	5
F0219	Pg 10 mg/mL, 57 mM (1%) Ala , 35 mM NaCl, 10 mM NaPi, pH 7.2	12.5	4	5,713	9

Example 9: Total percentage of solid and osmolality

[0094] The osmolality of several formulations of 10 mg/mL of plasminogen and the percentage of solid matters used to prepare said formulations are reported in Table 12. The osmolality of several formulations of 20 mg/mL of plasminogen and the percentage of solid matters used to prepare said formulations are reported in Table 13. The resulting osmolality of the tested formulations are advantageously falling within or very close to the physiological range of osmolality. The total percentage of solid matters is calculated from the content of plasminogen, tonicity modifier and bulking agent therein and is a good indicator of the size of the resulting cake after lyophilisation.

Table 12

Assign Sample ID	Sample Description	Total % Solid	mOsm/kg H ₂ O
Control	Pg Bulk (10 mg/ml) in 10 mM Na Citrate, 35 mM NaCl, pH 6.5	1.0%	104
F0468	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5mM (0.5 %) Arginine	1.5	157
F0469	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine, 54 mM (1%) Mannitol	2.5	209
F0470	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine, 108 mM (2%) Mannitol	3.5	377
F0471	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM	2.5	205

Assign Sample ID	Sample Description	Total % Solid	mOsm/kg H ₂ O
	NaCl, pH 6.5, 28.5 mM (0.5%) Arginine, 54 mM (1%) Sorbitol		
F0472	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine, 108 mM (2%) Sorbitol	3.5	298
F0473	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine	2.0	204
F0474	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine, 54 mM (1%) Mannitol	3.0	263
F0475	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine, 108 mM (2%) Mannitol	4.0	325
F0476	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine, 54 mM (1%) Sorbitol	3.0	252
F0477	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine, 108 mM (2%) Sorbitol	4.0	294
Assign Sample ID	Sample Description	Total % Solid	mOsm/kg H ₂ O
Control	Pg Bulk (10 mg/ml) in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2	1.0%	106
F0448	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine	1.5	155
F0449	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, 54 mM (1%) Mannitol	2.5	248
F0450	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, 108 mM (2%) Mannitol	3.5	345
F0451	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, 54 mM (1%) Sorbitol	2.5	235
F0452	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, 108 mM (2%) Sorbitol	3.5	374
F0453	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine	2.0	283
F0454	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35	3.0	304

Assign Sample ID	Sample Description	Total % Solid	mOsm/kg H ₂ O
	mM NaCl, pH 7.2, 57 mM (1%) Arginine, 54 mM (1%) Mannitol		
F0455	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57mM (1%) Arginine, 108 mM (2%) Mannitol	4.0	255
F0456	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine, 54 mM (1%) Sorbitol	3.0	277
F0457	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine, 108 mM (2%) Sorbitol	4.0	284
Assign Sample ID	Sample Description	Total % Solid	mOsm/kg H ₂ O
Control	Pg Bulk (10 mg/ml) in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0	1.00%	86
F0488	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine	1.5	144
F0489	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine, 54 mM (1%) Mannitol	2.5	176
F0490	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine, 108 mM (2%) Mannitol	3.5	324
F0491	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine, 54 mM (1%) Sorbitol	2.5	200
F0492	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine, 108 mM (2%) Sorbitol	3.5	199
F0493	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine	2.0	186
F0494	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine, 54 mM (1%) Mannitol	3.0	248
F0495	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine, 108 mM (2%) Mannitol	4.0	277
F0496	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine, 54 mM (1%) Sorbitol	3.0	239

Assign Sample ID	Sample Description	Total % Solid	mOsm/kg H ₂ O
F0497	Pg (10 mg/ml) Bulk P2146 in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine, 108 mM (2%) Sorbitol	4.0	290

Table 13

Assign Sample ID	Sample Description	Total % Solid	mOsm/kg H ₂ O
Control	Pg Bulk (20 mg/ml) in 10 mM Na Citrate, 35 mM NaCl, pH 6.5	2.0%	112
F0478	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine	2.5	170
F0479	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine, 54 mM (1%) Mannitol	3.5	245
F0480	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine, 108 mM (2%) Mannitol	4.5	307
F0481	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine, 54 mM (1%) Sorbitol	3.5	255
F0482	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine, 108 mM (2%) Sorbitol	4.5	306
F0483	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine	3.0	243
F0484	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine, 54 mM (1%) Mannitol	4.0	264
F0485	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine, 108 mM (2%) Mannitol	5.0	341
F0486	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine, 54 mM (1%) Sorbitol	4.0	337
F0487	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine, 108 mM (2%) Sorbitol	5.0	370

Assign Sample ID	Sample Description	Total % Solid	mOsm/kg H ₂ O
Control	Pg Bulk P24129 (20 mg/ml) in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2	2.0%	113
F0458	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine	2.5	200
F0459	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, 54 mM (1%) Mannitol	3.5	265
F0460	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, 108 mM (2%) Mannitol	4.5	293
F0461	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, 54 mM (1%) Sorbitol	3.5	279
F0462	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, 108 mM (2%) Sorbitol	4.5	310
F0463	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine	3.0	259
F0464	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine, 54 mM (1%) Mannitol	4.0	350
F0465	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine, 108 mM (2%) Mannitol	5.0	412
F0466	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine, 54 mM (1%) Sorbitol	4.0	366
F0467	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine, 108 mM (2%) Sorbitol	5.0	419
Assign Sample ID	Sample Description	Total % Solid	mOsm/kg H ₂ O
Control	Pg Bulk (20 mg/ml) in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0	2.0%	89
F0498	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine	2.5	201
F0499	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine, 54 mM (1%) Mannitol	3.5	260
F0500	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM	4.5	313

Assign Sample ID	Sample Description	Total % Solid	mOsm/kg H ₂ O
	NaCl, pH 8.0, 28.5 mM (0.5%) Arginine, 108 mM (2%) Mannitol		
F0501	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine, 54 mM (1%) Sorbitol	3.5	200
F0502	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine, 108 mM (2%) Sorbitol	4.5	319
F0503	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine	3.0	246
F0504	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine, 54 mM (1%) Mannitol	4.0	314
F0505	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine, 108 mM (2%) Mannitol	5.0	310
F0506	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine, 54 mM (1%) Sorbitol	4.0	282
F0507	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine, 108 mM (2%) Sorbitol	5.0	279

Example 10: Particle counts (PMC)

[0095] The particle counts (PMC) for particles equal or greater than 10 µm of the formulations described in Table 12 are reported in Table 14; and the particle counts (PMC) for particles equal or greater than 10µm of the formulations described in Table 13 are reported in Table 15.

Table 14

Assign Sample ID	Sample Description	Pre-Lyo PMC Count/mL 10 µm	Lyo-Recon PMC Count/mL 10 µm
Control	Pg Bulk (10 mg/ml) in 10 mM Na Citrate, 35 mM NaCl, pH 6.5	379	389
F0468	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine	35	224
F0469	Pg (10 mg/ml) Bulk in 10 mM Na	37	136

Assign Sample ID	Sample Description	Pre-Lyo PMC Count/mL 10 µm	Lyo-Recon PMC Count/mL 10 µm
	Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine, 54 mM (1%) Mannitol		
F0470	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine, 108 mM (2%) Mannitol	33	152
F0471	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine, 54 mM (1%) Sorbitol	21	159
F0472	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine, 108 mM (2%) Sorbitol	94	258
F0473	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine	45	342
F0474	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine, 54 mM (1%) Mannitol	61	218
F0475	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine, 108 mM (2%) Mannitol	129	138
F0476	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine, 54 mM (1%) Sorbitol	112	401
F0477	Pg (10 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine, 108 mM (2%) Sorbitol	155	159
Assign Sample ID	Sample Description	Pre-Lyo PMC Count/mL 10µm	Lyo-Recon PMC Count/mL 10µm
Control	Pg Bulk (10 mg/ml) in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2	2,400	3,819
F0448	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine	135	198
F0449	Pg (10 mg/ml) Bulk in 10 mM Na	171	104

Assign Sample ID	Sample Description	Pre-Lyo PMC Count/mL 10µm	Lyo-Recon PMC Count/mL 10µm
	Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, 54 mM (1%) Mannitol		
F0450	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, 108 mM (2%) Mannitol	267	254
F0451	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, 54 mM (1%) Sorbitol	122	133
F0452	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, 108 mM (2%) Sorbitol	214	176
F0453	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine	37	262
F0454	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine, 54 mM (1%) Mannitol	63	84
F0455	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine, 108 mM (2%) Mannitol	109	150
F0456	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine, 54 mM (1%) Sorbitol	86	169
F0457	Pg (10 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine, 108 mM (2%) Sorbitol	85	229
Control	Pg Bulk (10 mg/ml) in 10 mM Tris- HCl, 35 mM NaCl, pH 8.0	3,341	4,007
F0488	Pg (10 mg/ml) Bulk in 10 mM Tris- HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine	150	414
F0489	Pg (10 mg/ml) Bulk in 10 mM Tris- HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine, 54 mM (1%)	151	387

Assign Sample ID	Sample Description	Pre-Lyo PMC Count/mL 10µm	Lyo-Recon PMC Count/mL 10µm
	Mannitol		
F0490	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine, 108 mM (2%) Mannitol	91	528
F0491	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine, 54 mM (1%) Sorbitol	240	589
F0492	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine, 108 mM (2%) Sorbitol	120	507
F0493	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine	103	229
F0494	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine, 54 mM (1%) Mannitol	90	507
F0495	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine, 108 mM (2%) Mannitol	67	256
F0496	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine, 54 mM (1%) Sorbitol	131	375
F0497	Pg (10 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine, 108 mM (2%) Sorbitol	62	456

Table 15

Assign Sample ID	Sample Description	Pre-Lyo PMC Count/mL 10 µm	Lyo-Recon PMC Count/mL 10 µm
Control	Pg Bulk (20 mg/ml) in 10 mM Na Citrate, 35 mM NaCl, pH 6.5	1100	793
F0478	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine	171	358
F0479	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine, 54 mM (1%) Mannitol	118	572

Assign Sample ID	Sample Description	Pre-Lyo PMC Count/mL 10 µm	Lyo-Recon PMC Count/mL 10 µm
F0480	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine, 108 mM (2%) Mannitol	132	515
F0481	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine, 54 mM (1%) Sorbitol	96	427
F0482	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 28.5 mM (0.5%) Arginine, 108 mM (2%) Sorbitol	101	585
F0483	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine	132	286
F0484	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine, 54 mM (1%) Mannitol	115	537
F0485	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine, 108 mM (2%) Mannitol	154	362
F0486	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine, 54 mM (1%) Sorbitol	114	535
F0487	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57 mM (1%) Arginine, 108 mM (2%) Sorbitol	194	384
Assign Sample ID	Sample Description	Pre-Lyo PMC Count/mL 10 µm	Lyo-Recon PMC Count/mL 10 µm
Control	Pg Bulk (20 mg/ml) in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2	3,784	2,619
F0458	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine	75	398
F0459	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, 54 mM (1%) Mannitol	96	193

Assign Sample ID	Sample Description	Pre-Lyo PMC Count/mL 10 µm	Lyo-Recon PMC Count/mL 10 µm
F0460	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, 108 mM (2%) Mannitol	128	198
F0461	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, 54 mM (1%) Sorbitol	123	659
F0462	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 28.5 mM (0.5%) Arginine, 108 mM (2%) Sorbitol	103	317
F0463	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine	93	270
F0464	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine, 54 mM (1%) Mannitol	99	386
F0465	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine, 108 mM (2%) Mannitol	139	172
F0466	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine, 54 mM (1%) Sorbitol	67	681
F0467	Pg (20 mg/ml) Bulk in 10 mM Na Phosphate, 35 mM NaCl, pH 7.2, 57 mM (1%) Arginine, 108 mM (2%) Sorbitol	93	261
Assign Sample ID	Sample Description	Pre-Lyo PMC Count/ml 10 µm	Lyo-Recon PMC Count/mL 10 µm
Control	Pg Bulk (20 mg/ml) in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0	6,315	7,123
F0498	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine	629	3,382
F0499	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine, 54	1329	1,596

Assign Sample ID	Sample Description	Pre-Lyo PMC Count/ml 10 μ m	Lyo-Recon PMC Count/mL 10 μ m
	mM (1%) Mannitol		
F0500	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine, 108 mM (2%) Mannitol	1007	896
F0501	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine, 54 mM (1%) Sorbitol	649	1,401
F0502	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 28.5 mM (0.5%) Arginine, 108 mM (2%) Sorbitol	793	684
F0503	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine	459	703
F0504	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine, 54 mM (1%) Mannitol	505	608
F0505	Pg (20 mg/ml) Bulk P2147 in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine, 108 mM (2%) Mannitol	155	382
F0506	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine, 54 mM (1%) Sorbitol	213	898
F0507	Pg (20 mg/ml) Bulk in 10 mM Tris-HCl, 35 mM NaCl, pH 8.0, 57 mM (1%) Arginine, 108 mM (2%) Sorbitol	101	431

[0096] Pictures of vials are shown in Figures 12, 13 and 14, which contains formulations F0498, F0449 and F0459, respectively, as representative examples of the appearance of the lyophilized formulations reported in Tables 12-15.

Example 10: Reversible plasminogen aggregation

[0097] Plasminogen aggregation is reversible as demonstrated by this study. Frozen

Plasminogen samples (Pg Bulk) 5 mg/ml, dialyzed in dH₂O and heavily precipitated, were thawed, filtered through 1.2 µm syringe filter, and used as starting material for control (Pg control). Aliquots of 2 ml Pg control were added to glass vials, pre-spiked with buffer stocks of 1 M Na Citrate pH 6.5 (Sample 1), 0.5 M Na Phosphate pH 7.2 (Sample 2) or 1 M Tris-HCl pH 8.0 (Sample 3) to obtain final 10 mM buffer concentration at each pH, respectively. Samples 1, 2 and 3 are shown in Figure 15, where the non-spiked Pg control is Sample 0. Samples 1, 2 and 3 were further spiked with stock solutions of 5 M NaCl and 0.8 M Arginine to obtain a final concentration of 35 mM NaCl and 28.5 mM (0.5%) Arg in each sample and are shown in Figure 16. Turbidity standards of 0.02, 20, 100, 800 NTU respectively, were used in Figures 15 and 16, as reference. It can be noted from Figure 15 that pH adjustment has dissolved a considerable amount of aggregates. In Figure 16, the Samples 1, 2 and 3 are rendered transparent by the addition of Arginine and NaCl and no aggregate can be visually detected.

[0098] The turbidity of Samples 0, 1, 2 and 3 of Figure 16 were measured by reading the Optical Density (OD) at 550 nm using standard calibration with turbidity reference standards shown in Figures 15 and 16. The turbidity is reported in Nephelometric Turbidity Units (NTU) in Table 16. Aliquots of 1 ml of each sample was measured before (T=0) and after spiking the samples 1, 2 and 3 with buffer, NaCl and Arginine at 0.1 hour, 17 hours, 21 hours and 24 hours. Disaggregation is rapid as it can be noted at only 0.1 hour after spiking, and it is maintained over 24 hours.

Table 16

Assigned Sample ID	Sample Description	Turbidity (NTU by OD 550)				
		T=0	T=0.1h	T=17h	T=21h	T=24h
F0138	Pg Bulk (5 mg/ml), Conc. 2x dH ₂ O exchanged	1008	1008	1209	858	372
F0139	Pg Bulk (5 mg/ml) in 10 mM Sodium Citrate, 35 mM NaCl, 28.5 mM (0.5%) Arginine, pH 6.5	1008	30	37	39	60
F0140	Pg Bulk (5 mg/ml) in 10 mM Sodium Phosphate, 35 mM NaCl, 28.5 mM (0.5%) Arginine, pH 7.2	1008	49	60	68	100
F0141	Pg Bulk (5 mg/ml) in 10 mM Tris, 35 mM NaCl, 28.5 mM (0.5%) Arginine, pH 8.0	1008	49	56	66	92

Example 11: Effect of Amino Acid over Time on Composition Turbidity

[0099] Turbidity has been measured in compositions containing 10 mg plasminogen and 35 mM NaCl with 0.5% glycine, arginine, alanine and without amino acid, at pH 6.5 in sodium

citrate buffer (Figure 17), pH 7.2 in sodium phosphate buffer (Figure 18), and at pH 8.0 in Tris buffer (Figure 19).

[0100] The compositions tested in Figures 17, 18 and 19 were reproduced in presence of 0.5% mannitol as a bulking agent, and reported in Figures 20, 21 and 22, respectively.

[0101] In all cases, the presence of the amino acid provides a stabilizing effect and maintain or lower the turbidity. Comparing the type of amino acids, arginine shows the best stabilizing effect and lower the turbidity at all tested pH and in presence and absence of bulking agent, i.e. 0.5% mannitol.

Example 12: composition of elevated concentration of plasminogen

[0102] Compositions containing a high concentration of plasminogen have been studied. The PMC counts for particles of 10 μm or greater, and of 25 μm or greater have been reported in Table 17 for compositions of 20 mg/ml plasminogen in 10 mM or 100 mM sodium citrate, 35 mM NaCl, at pH6.5, with and without 1% arginine, and compositions of 37 and 56 mg/ml plasminogen in 100 mM sodium citrate, 35 mM NaCl, at pH6.5, with 1% arginine.

Table 17 shows that the formulations of the present invention advantageously allow the preparation of compositions comprising elevated concentrations of plasminogen (37 and 56 mg/ml) without causing unacceptable level of particles.

Table 17

Assign Sample ID	Sample Description	PMC_Count/Vial	
		10 μm	25 μm
P24138	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5	983	8
F0483	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 35 mM NaCl, pH 6.5, 57mM (1%) Arginine	525	0
F0614	Pg (20 mg/ml) Bulk in 10 mM Na Citrate, 100 mM NaCl, pH 6.5, 57mM (1%) Arginine	667	33
F0615	Pg (37 mg/ml) Bulk in 10 mM Na Citrate, 100 mM NaCl, pH 6.5, 57mM (1%) Arginine	325	33
F0616	Pg (56 mg/ml) Bulk in 10 mM Na Citrate, 100 mM NaCl, pH 6.5, 57mM (1%) Arginine	292	17

Example 13: Plasminogen activity

[0103] The plasminogen activity for each formulation exemplified herein was tested and compared to the plasminogen activity of the initial preparation or bulk preparation (Pg bulk). It

has been noted that the plasminogen activity is not affected by any of these formulations.

REFERENCES CITED IN THE DESCRIPTION

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Patentkrav

1. Farmaceutisk sammensætning, der omfatter:

- plasminogen eller en biologisk aktiv variant deraf med mindst 90 % aminosyresekvensidentitet med et nativt plasminogenpolypeptid, i en koncentration fra ca. 0,01 mg/ml til ca. 80 mg/ml;
- en tonicitetsmodifikator, der forekommer i en koncentration, som er indstillet til nå en osmolalitet for sammensætningen på mellem ca. 180 mOsm og ca. 350 mOsm, hvor tonicitetsmodifikatoren er natriumchlorid og forekommer i en koncentration på mellem ca. 30 mM og ca. 100 mM; og
- et stabiliseringsmiddel, der er arginin eller glycin, i en koncentration fra ca. 10 mM til ca. 100 mM;

hvor sammensætningen har en pH-værdi fra ca. 5,0 til ca. 8,0, og

hvor plasminogenet eller en biologisk aktiv variant deraf består af mere end 80 % Glu-plasminogen, eller plasminogenet eller en biologisk aktiv variant deraf er Glu-plasminogen, hvor udtrykket "ca." dækker + eller - 10 % af den tilsvarende værdi.

2. Farmaceutisk sammensætning ifølge krav 1, hvor pH-værdien er fra ca. 6,0 til ca. 8,0; eller fra ca. 6,5 til ca. 7,5, hvor udtrykket "ca." dækker + eller - 10 % af den tilsvarende værdi.

3. Farmaceutisk sammensætning ifølge et hvilket som helst af kravene 1 til 2, hvor sammensætningen indeholder en mængde partikler, der er lig med eller større end 10 µm, hvilket er mindre end 6000 partikler pr. beholder, hvor beholderen har et nominelt indhold på mindre end eller lig med 100 ml.

4. Farmaceutisk sammensætning ifølge krav 3, hvor mængden af partikler er mindre end 2000 eller 1000 partikler pr. beholder, hvor beholderen har et nominelt indhold på mindre end eller lig med 100 ml.

5. Farmaceutisk sammensætning ifølge et hvilket som helst foregående krav, der endvidere omfatter et konserveringsmiddel; eventuelt hvor konserveringsmidlet er m-cresol, benzylalkohol, methanol, ethanol, iso-propanol, butylparaben, ethylparaben,

methylparaben, phenol, glycerol, xylitol, resorcinol, catechol, 2,6-dimethylcyclohexanol, 2-methyl-2,4-pentadiol, dextran, polyvinylpyrrolidon, 2-chlorphenol, benzethoniumchlorid, merthiolat (thimersosal), benzoesyre (propylparaben) MW 180,2, benzoesyre MW 122,12, benzalkoniumchlorid, chlorbutanol, natriumbenzoat, natriumpropionat, cetylpyridiniumchlorid eller en hvilken som helst kombination deraf; og eventuelt hvor koncentrationen af konserveringsmidlet udgør fra ca. 0,005 til ca. 10 % (vægt/vol), hvor udtrykket "ca." dækker + eller - 10 % af den tilsvarende værdi.

6. Farmaceutisk sammensætning ifølge et hvilket som helst foregående krav, der er egnet til intravenøs, subkutan, topisk, intradermal, oftalmisk og/eller intramuskulær administration.

7. Farmaceutisk sammensætning ifølge et hvilket som helst foregående krav, hvor stabiliseringsmidlet er i en koncentration på ca. 25 mM til ca. 75 mM, hvor udtrykket "ca." dækker + eller - 10 % af den tilsvarende værdi.

8. Farmaceutisk sammensætning ifølge et hvilket som helst foregående krav, hvor koncentrationen af plasminogenet eller den biologisk aktive variant deraf udgør fra ca. 2 mg/ml til ca. 20 mg/ml, hvor udtrykket "ca." dækker + eller - 10 % af den tilsvarende værdi.

9. Farmaceutisk sammensætning ifølge krav 8, hvor koncentrationen af plasminogenet eller den biologisk aktive variant deraf udgør ca. 20, 10 eller 5 mg/ml, hvor udtrykket "ca." dækker + eller - 10 % af den tilsvarende værdi.

10. Farmaceutisk sammensætning ifølge et hvilket som helst foregående krav, hvor plasminogenet eller den biologisk aktive variant deraf udgør mindst 80 % af sammensætningens totale proteinindhold, mere end 90 % af sammensætningens totale proteinindhold, mere end ca. 95 % af sammensætningens totale proteinindhold eller mere end ca. 98 % af sammensætningens totale proteinindhold, hvor udtrykket "ca." dækker + eller - 10 % af den tilsvarende værdi.

11. Farmaceutisk sammensætning ifølge et hvilket som helst foregående krav, hvor tonicitetsmodifikatoren forekommer i en koncentration på ca. 25 mM til ca. 50 mM eller på ca. 35 mM, hvor udtrykket "ca." dækker + eller - 10 % af den tilsvarende værdi.

12. Farmaceutisk sammensætning ifølge et hvilket som helst foregående krav, der yderligere omfatter en ikke-reducerende sukkerart.

13. Farmaceutisk sammensætning ifølge et hvilket som helst foregående krav, hvor plasminogenet eller en biologisk aktiv variant deraf er humant plasminogen.

14. Farmaceutisk sammensætning ifølge et hvilket som helst foregående krav til anvendelse som et medikament.

DRAWINGS

Drawing

Figure 1A

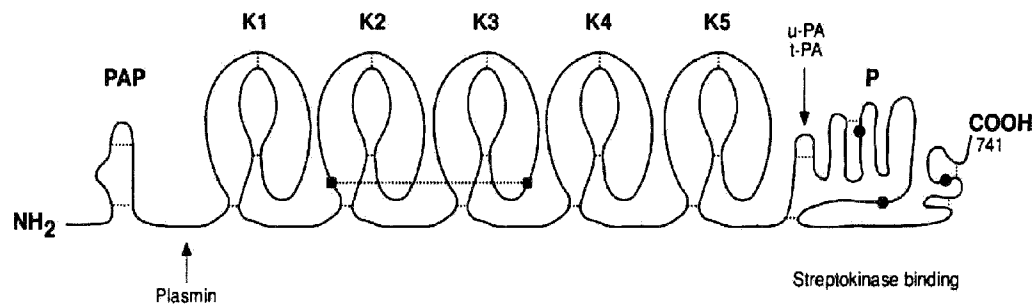


Figure 1B

Human plasminogen precursor (UniProt accession # P00747)

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Figure 2

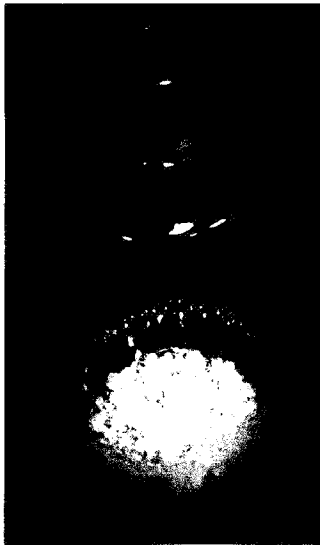


Figure 3

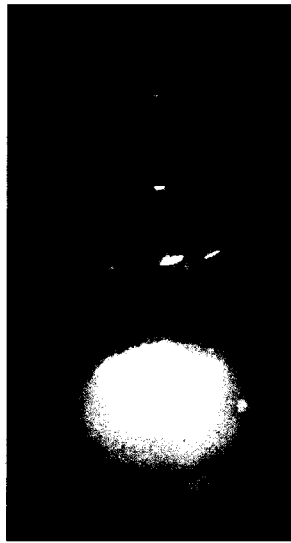


Figure 4



Figure 5

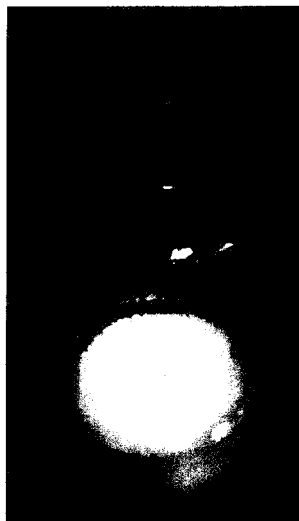


Figure 6



Figure 7

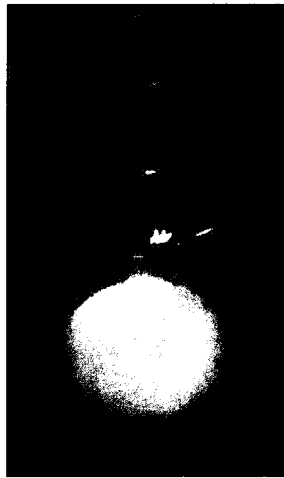


Figure 8

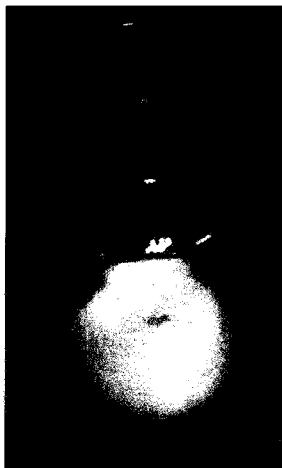


Figure 9

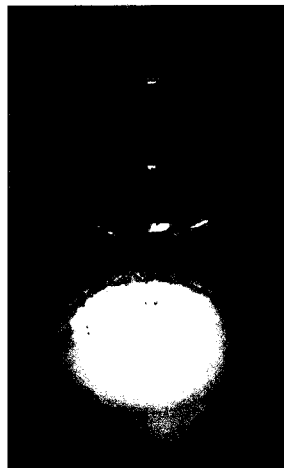


Figure 10



Figure 11

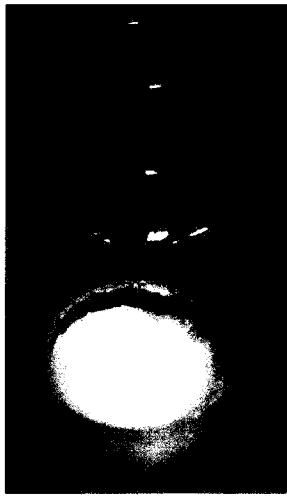


Figure 12



Figure 13



Figure 14



Figure 15

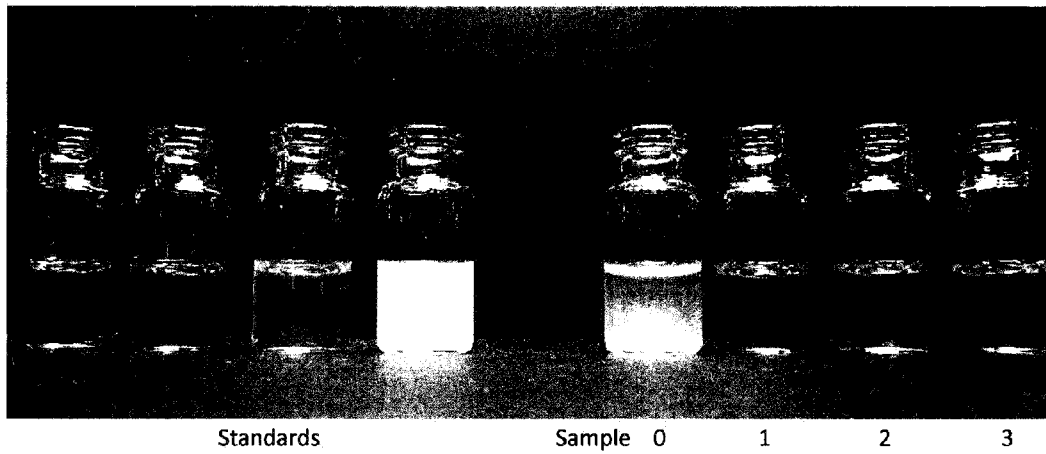


Figure 16

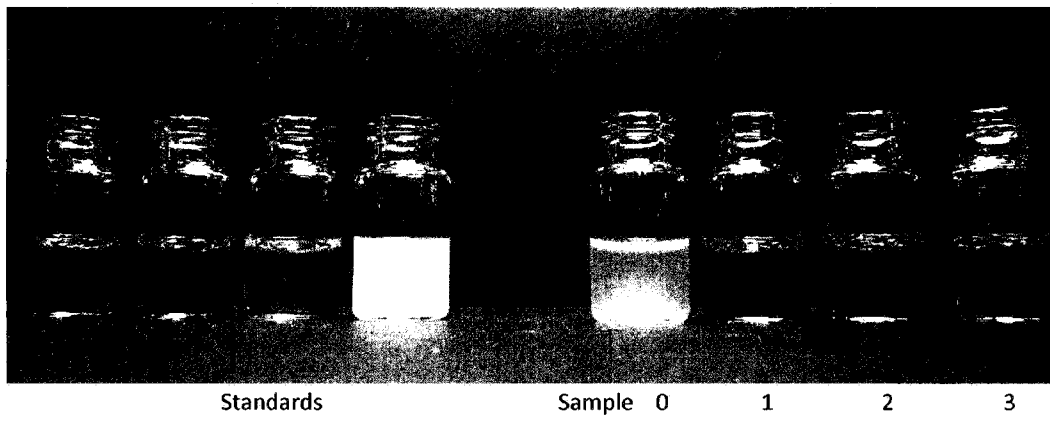


Figure 17

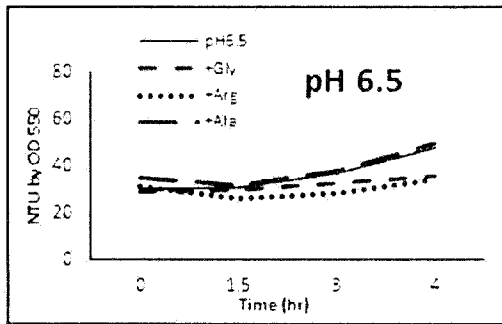


Figure 18

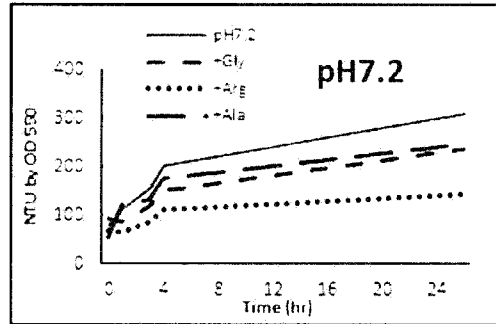


Figure 19

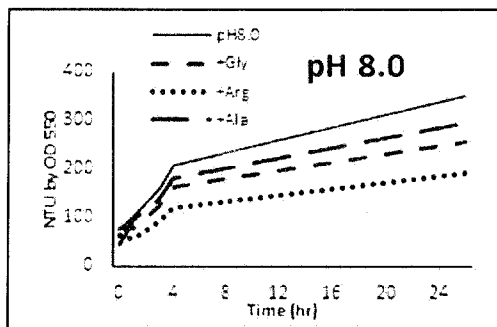


Figure 20

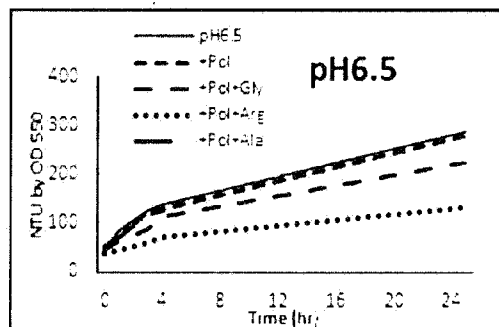


Figure 21

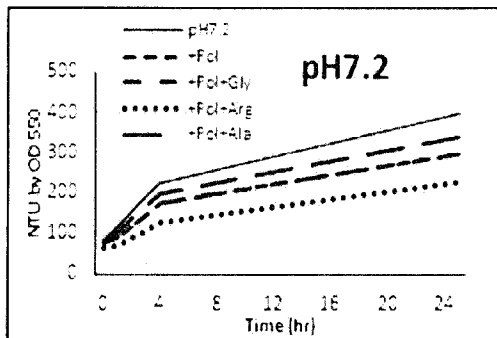


Figure 22

