PHARMACEUTICAL COMPOSITION

FIELD OF THE INVENTION

The present invention belongs to the field of pharmaceutical formulations. More specifically, it relates to a pharmaceutical composition, liquid or freeze-dried, comprising an antibody, sucrose, an amino acid or a mixture of amino acids and a surfactant.

BACKGROUND OF THE INVENTION

The industrial manufacture of therapeutic antibodies is a complex task. It requires scale-up from processes developed in a laboratory for producing antibodies in milligram quantities to multi-kilogram quantities. Despite antibodies being fairly stable molecules, when stored at high concentration over a period of time they may, nevertheless, suffer from chemical and physical instability. Typical chemical instability may result in deamidation, hydrolysis, oxidation, beta-elimination, disulfide exchange or reduction. Physical instability can result in denaturation, aggregation or precipitation.

Whilst liquid formulations can be administered with minimal preparation, they have the drawback of being less stable over time. Liquid water-based formulations are particularly complex; water acts as a reactant or facilitates the transfer of reactants leading to chemical degradation and protein instability. The stabilization of proteins in liquid formulations to avoid or minimize aggregation, precipitation or degradation remains a particular challenge. Aggregation, with the formation of insoluble matter or precipitate, is a particular problem. This may cause various problems such as aggregates' formation which could lead to immunological reactions upon administration and/or difficulty in performing proper administration of the pharmaceutical formulation, e.g. by causing blockage of the delivery device.

Antibodies may be formulated in freeze-dried, i.e. lyophilized form for reconstitution in a solvent shortly before administration. Freeze-dried formulations of antibodies tend to be more stable than liquid water-based formulation. Freeze-drying facilitates achieving high concentrations of the antibody in the final formulation, thus, reducing the injection volume as well as the injection time.

In addition, freeze-drying (lyophilization) is the method of choice for long term storage stabilization of monoclonal antibodies that are otherwise unstable in liquid form. Freeze-drying

antibodies at a lower temperature reduces the damage to the products and aids retaining molecular integrity. It extends the shelf life, reduces the temperature requirement for shipping and preserves the antibodies chemical and biological properties.

Antibodies, as other proteins, undergo denaturation upon freeze-drying in the absence of stabilizers. Different stabilizers such as sugars or polyols are normally added to the formulations to protect antibodies against degradation during freeze-drying and storage. The level of stabilization afforded by sugars or polyols generally depends on their concentrations. Increasing sugar/polyol concentration to a certain level may eventually reach a limit of stabilization or even destabilize a protein during freeze-drying, so a specific concentration of stabilizer to protein is required for storage stability of a freeze-dried antibody (Chang L, et al. J Pharm Sci. 2005; 94:1445-55). The level of stabilizers used for protection during freeze-drying depends on the formulation composition, concentration and physical properties of the stabilizer, and its compatibility with the antibody. The mechanism of stabilization during drying is that the stabilizer acts as a water substitute. The interaction between water and proteins is critical to the conformational stability of the proteins. When water is removed during drying, the stabilizers can form hydrogen bonds with the protein, as water molecules do, thereby preserve the native protein structure during lyophilization process (Chang L, et al. J Pharm Sci. 2005; 94:1445-55).

Despite lyophilization being a good choice to achieve the desired long term storage for antibodies, there is no simple, universal protocol in formulating antibodies. Antibodies can become unstable during lyophilization processes and/or long term storage, aggregates may form upon reconstitution or lyophilized cakes may take too long to reconstitute. High concentration of stabilizers may affect the physic-chemical properties (i.e. viscosities) of the final formulation.

Given the above, there remains a need in the art to provide further improved pharmaceutical compositions of antibodies suitable for freeze-drying.

SUMMARY OF THE INVENTION

The present invention addresses the above-identified need by providing pharmaceutical compositions comprising an antibody or a fragment thereof suitable for freeze-drying (i.e. lyophilizing). The resulting pharmaceutical compositions can be provided as freeze-dried (i.e. lyophilized) compositions which can be reconstituted into a solvent at the time of use or as reconstituted liquid formulations ready for administration. Reconstitution in reduced volumes

allows achieving formulation with high concentrations of antibody or fragment thereof with a low level of aggregation and delivery time.

The following specific embodiments are described as numbered hereinafter:

1. A pharmaceutical composition comprising:
    a. an antibody or an antigen-binding fragment thereof;
    b. from 1% to 20% w/v sucrose;
    c. an amino acid or a mixture of amino acids; and
    d. a surfactant.
2. The pharmaceutical composition according to Embodiment 1, wherein having pH of from 4.0 to 7.5.
3. The pharmaceutical composition according to Embodiment 1 or Embodiment 2, wherein the antibody or antigen-binding fragment thereof is at a concentration of from 1 to 200 mg/mL, preferably from 50 to 150 mg/mL, more preferably from 80 to 140mg/mL.
4. The pharmaceutical composition according to any one of the preceding Embodiments, wherein the composition comprises from 5% to 13% w/v sucrose, preferably from 7% to 10% w/v sucrose, more preferably 7% w/v sucrose.
5. The pharmaceutical composition according to any one of the preceding Embodiments, wherein:
    a. the amino acid is histidine or proline; or
    b. the mixture of amino acids comprises histidine and proline.
6. The pharmaceutical composition according to any one of the preceding Embodiments, wherein the composition comprises 0.01 to 1.0%w/v, preferably from 0.01% to 0.5% w/v of the surfactant.
7. The pharmaceutical composition according to Embodiment 6, wherein the surfactant is polysorbate 80.
8. The pharmaceutical composition according to any one of the preceding Embodiments, wherein the antibody or antigen-binding fragment thereof specifically binds human FcRn.
9. The pharmaceutical composition according to any one of the preceding Embodiments, wherein the antibody is a human or humanised antibody.
10. The pharmaceutical composition according to any one of the preceding Embodiments, wherein the antibody antigen-binding fragment thereof comprises a light variable region as defined in SEQ ID NO: 7 and a heavy variable region as defined in SEQ ID NO: 8.

11. The pharmaceutical composition according to any one of the preceding Embodiments, wherein the composition comprises from 1mg/mL to 200mg/mL of antibody or antigen-binding fragment thereof, from 10 mM to 50mM histidine, from 10 to 300mM proline, from 1% to 20% w/v sucrose, from 0.01% to 1.0% w/v polysorbate 80, at pH 5.6; preferably the composition comprises from 50mg/mL to 150mg/mL of antibody or antigen-binding fragment thereof, from 20 mM to 40mM histidine, from 50 to 280mM proline, from 5% to 13% w/v sucrose, from 0.01% to 0.5% w/v polysorbate 80, at pH 5.6; more preferably the composition comprises from 80mg/mL to 140mg/mL of antibody or antigen-binding fragment thereof, 30 mM histidine, 180mM proline, 7% w/v sucrose, 0.03% w/v polysorbate 80, at pH 5.6; and even more preferably the composition comprises 100mg/mL of antibody or antigen-binding fragment thereof, 30 mM histidine, 180mM proline, 7% w/v sucrose, 0.03% w/v polysorbate 80, at pH 5.6.
12. The pharmaceutical composition according to any one of the preceding Embodiments, wherein the composition further comprises mannitol and/or arginine and/or glycine.
13. A freeze-dried pharmaceutical composition comprising an antibody or an antigen-binding fragment thereof, sucrose, an amino acid or a mixture of amino acids, and optionally a surfactant.
14. The freeze-dried formulation according to Embodiment 13 wherein the formulation comprises from 10% to 40% w/w sucrose, preferably from 20% to 40% w/w sucrose.
15. A freeze-dried formulation comprising an antibody comprising a light variable region as defined in SEQ ID NO: 7 and a heavy variable region as defined in SEQ ID NO: 8 and from 10% to 40% w/w sucrose.
16. A liquid pharmaceutical formulation comprising the pharmaceutical composition according to any one of the Embodiments 13 to 15 and a solvent.
17. The liquid pharmaceutical formulation according to Embodiment 16, wherein the solvent is water.
18. A container comprising the freeze-dried formulation according to any one of Embodiments 13 to 15 or the liquid pharmaceutical formulation according to any one of Embodiments 16 or 17.
19. The pharmaceutical composition according to any one of Embodiments 1 to 12, the freeze-dried formulation according to any one of Embodiments 13 to 15 or the liquid

pharmaceutical formulation according to any one of Embodiments 16 to 17 for use in therapy.

20. The pharmaceutical composition according to any one of Embodiments 1 to 12, the freeze-dried formulation according to any one of Embodiment 13 to 15 or the liquid pharmaceutical formulation according to any one of Embodiments 16 to 17 for use in the treatment of inflammatory or autoimmune diseases selected from myasthenia gravis, Pemphigus vulgaris, Neuromyelitis optica, Guillain-Barré syndrome, lupus, and thrombotic thrombocytopenic purpura, Idiopathic thrombocytopenic purpura (ITP), Chronic inflammatory demyelinating polyneuropathy (CIDP) and combinations thereof.
21. A method for treating an inflammatory or autoimmune disease in a mammalian subject comprising administering pharmaceutical composition according to any one of Embodiments 1 to 12, the freeze-dried formulation according to any one of Embodiment 13 to 15 or the liquid pharmaceutical formulation according to any one of Embodiments 16 to 17; wherein the inflammatory or autoimmune disease is selected from myasthenia gravis, Pemphigus vulgaris, Neuromyelitis optica, Guillain-Barré syndrome, lupus, and thrombotic thrombocytopenic purpura, Idiopathic thrombocytopenic purpura (ITP), Chronic inflammatory demyelinating polyneuropathy (CIDP) and combinations thereof.
22. A method for producing a pharmaceutical composition comprising an anti-FcRn antibody, wherein the method comprises the steps of:
    (i) preparing a composition comprising an anti-FcRn antibody; and
    (ii) adding 1% to 20% w/v sucrose.
23. The method according to Embodiment 22 wherein the pharmaceutical composition resulting from step (ii) is a pharmaceutical composition according to any one of Embodiments 1 to 12.
24. The method according to Embodiment 22 or Embodiment 23, wherein the method further comprises the step of freeze-drying the pharmaceutical composition resulting from step (ii).
25. The pharmaceutical composition obtained by the method according to Embodiment 24.
26. A method for reconstituting a pharmaceutical composition according to any one of Embodiments 13, 14, 15, 24 or 25 by adding a solvent, wherein the solvent is preferably water.

27. A liquid pharmaceutical formulation obtained by the method according to Embodiment 26.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. **Reconstitution time upon lyophilisation**. Reconstitution time (recon time) is shown on the y-axis in minutes per each of the formulations (x-axis) of Table 2.

Figure 2. **Aggregates formation by SEC**. Total aggregates as % area are shown on the y-axis per each of the formulations (x-axis) of Table 2.

Figure 3. **HMW aggregate formation by DLS**. Intensities (as %) were plotted on the y-axis per each formulation (x-axis) of Table 2.

Figure 4. **Charge variants**. The formation of acidic (A) and basic (B) variants was plotted as a function of the %area (y-axis) per each formulation (x-axis) of Table 2.

Figure 5. **Viscosity**. The viscosity (cP) is shown on the y-axis per each formulation (x-axis) of Table 2.

Figure 6. **Osmolality**. The Osmolality (mOsm/kg) is shown on the y-axis per each formulation (x-axis) of Table 2.

Figure 7. **Reconstitution time upon lyophilisation**. Reconstitution time (recon time) is shown on the y-axis in minutes per each of the formulations (x-axis) of Table 5.

Figure 8. **Aggregates formation by SEC**. Total aggregates as % area are shown on the y-axis per each of the formulations (x-axis) of Table 5.

Figure 9. **HMW aggregate formation by DLS**. Masses (as %) were plotted on the y-axis per each formulation (x-axis) of Table 5.

Figure 10. **Charge variants**. The formation of acidic (A) and basic (B) variants was plotted as a function of the %area (y-axis) per each formulation (x-axis) of Table 5 comprising the anti-FcRn antibody at 100mg/mL.

Figure 11. **Charge variants**. The formation of acidic (A) and basic (B) variants was plotted as a function of the %area (y-axis) per each formulation (x-axis) of Table 5 comprising an anti-FcRn antibody at 140mg/mL.

Figure 12. **Viscosity**. The viscosity (cP) is shown on the y-axis per each formulation (x-axis) of Table 5.

Figure 13. **Osmolality**. The Osmolality (mOsm/kg) is shown on the y-axis per each formulation (x-axis) of Table 5.

DETAILED DESCRIPTION OF THE INVENTION

The pharmaceutical composition according to the invention comprises an antibody or an antigen binding fragment thereof; from 1% to 20% w/v sucrose; an amino acid or a mixture of amino acids; and a surfactant.

The concentration of antibody or antigen-binding fragment thereof in the pharmaceutical composition may be from 1 to 200 mg/mL, preferably from 50 to 200 mg/mL, more preferably from 80 to 140 mg/mL.

Preferably the antibody or antigen-binding fragment thereof comprised in the pharmaceutical composition according to the invention specifically binds to human FcRn.

The term "specifically binds to human FcRn", "specifically binding to human FcRn", and equivalents as used herein means the antibody will bind to human FcRn with sufficient affinity and specificity to achieve a biologically meaningful effect. The antibody selected will normally have a binding affinity for human FcRn, for example, the antibody may bind human FcRn with a Kd value of between 100nM and 1pM. Antibody affinities may be determined for example by a surface plasmon resonance bases assay, such as the BIAcore assay; enzyme-linked immunoabsorbent assay (ELISA); and competition assays (e.g. RIA's). Within the meaning of the present invention an antibody or antigen-binding fragment thereof specifically binding to human FcRn may also bind to another molecule, e.g. cyno FcRn or such as by way of a non-limiting example in the case of a bispecific antibody.

FcRn is a non-covalent complex of membrane protein FcRn α chain and β2 microglobulin (β2M). In adult mammals FcRn plays a key role in maintaining serum antibody levels by acting as a receptor that binds and salvages antibodies of the IgG isotype. IgG molecules are endocytosed by endothelial cells, and if they bind to FcRn, are recycled transcytosed out into, for example circulation. In contrast, IgG molecules that do not bind to FcRn enter the cells and are targeted to the lysosomal pathway where they are degraded. A variant IgG1 in which His435

is mutated to alanine results in the selective loss of FcRn binding and a significantly reduced serum half-life (Firan et al. 2001, International Immunology 13:993).

The antibody or antigen-binding fragment thereof binding specifically to human FcRn, preferably also neutralizes human FcRn.

The term “neutralizes” as used herein refers to an antibody that inhibits or substantially reduces the biological effect of the molecule to which it specifically binds. Therefore, the expression “the antibody neutralizes human FcRn” refers to an antibody that specifically binds to human FcRn and inhibits or substantially reduces the biological effect thereof such as by blocking FcRn binding to IgG.

The term "antibody" or "antibodies" as used herein refers to monoclonal or polyclonal antibodies and is not limited to recombinant antibodies that are generated by recombinant technologies as known in the art.

Preferably, the antibody comprised in the pharmaceutical composition according to the invention is a monoclonal antibody which specifically binds human FcRn.

"Antibody" or "antibodies" include antibodies' of any species, in particular of mammalian species, having two essentially complete heavy and two essentially complete light chains, human antibodies of any isotype, including IgA_1, IgA_2, IgD, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, IgG_4 IgE and IgM and modified variants thereof, non-human primate antibodies, e.g. from chimpanzee, baboon, rhesus or cynomolgus monkey, rodent antibodies, e.g. from mouse, rat or rabbit; goat or horse antibodies, and derivatives thereof, or of bird species such as chicken antibodies or of fish species such as shark antibodies. The term "antibody" or "antibodies" also refers to "chimeric" antibodies in which a first portion of at least one heavy and/or light chain antibody sequence is from a first species and a second portion of the heavy and/or light chain antibody sequence is from a second species. Chimeric antibodies of interest herein include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (e.g. Old World Monkey, such as baboon, rhesus or cynomolgus monkey) and human constant region sequences. "Humanized" antibodies are chimeric antibodies that contain a sequence derived from non-human antibodies. For the most part, humanized antibodies are human antibodies (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region or complementarity determining region (CDR) of a non-human species (donor antibody) such as mouse, rat, rabbit, chicken or non-human primate, having the desired specificity, affinity, and activity. In most instances

residues of the human (recipient) antibody outside of the CDR; i.e. in the framework region (FR), are additionally replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. Humanization reduces the immunogenicity of non-human antibodies in humans, thus facilitating the application of antibodies to the treatment of human diseases. Humanized antibodies and several different technologies to generate them are well known in the art. The term "antibody" or "antibodies" also refers to human antibodies, which can be generated as an alternative to humanization. For example, it is possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of production of endogenous murine antibodies. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies with specificity against a particular antigen upon immunization of the transgenic animal carrying the human germ-line immunoglobulin genes with said antigen. Technologies for producing such transgenic animals and technologies for isolating and producing the human antibodies from such transgenic animals are known in the art. Alternatively, in the transgenic animal; e.g. mouse, only the immunoglobulin genes coding for the variable regions of the mouse antibody are replaced with corresponding human variable immunoglobulin gene sequences. The mouse germline immunoglobulin genes coding for the antibody constant regions remain unchanged. In this way, the antibody effector functions in the immune system of the transgenic mouse and consequently the B cell development are essentially unchanged, which may lead to an improved antibody response upon antigenic challenge in vivo. Once the genes coding for a particular antibody of interest have been isolated from such transgenic animals the genes coding for the constant regions can be replaced with human constant region genes in order to obtain a fully human antibody. The term “antibody” or “antibodies” as used herein, also refers to an aglycosylated antibody.

The term "antigen-binding fragment thereof" or grammatical variations thereof as used herein refers to an antibody fragment. A fragment of an antibody comprises at least one heavy or light chain immunoglobulin domain as known in the art and binds to one or more antigen(s). Examples of antibody fragments according to the invention include Fab, Fab', $F(ab')_2$, and Fv and scFv fragments; as well as diabodies, triabodies, tetrabodies, minibodies, domain

antibodies(dAbs), such as sdAbs, VHH or camelid antibodies (e.g. from camels or llamas such as Nanobodies™) and VNAR fragments, single-chain antibodies, bispecific, trispecific, tetraspecific or multispecific antibodies formed from antibody fragments or antibodies, including but not limited to Fab-Fv or Fab-Fv-Fv constructs. Antibody fragments as defined above are known in the art.

The antibody or antigen-binding fragment thereof comprised in the pharmaceutical composition according to the invention may be a human or humanized antibody, preferably a humanized monoclonal antibody which specifically binds human FcRn.

More preferably the pharmaceutical composition according to the invention comprises (Table 1):

1) an antibody or antigen-binding fragment thereof which
    a. comprises CDR-H1 having the sequence as defined in SEQ ID NO:1; CDR-H2 having the sequence as defined in SEQ ID NO:2; CDR-H3 having the sequence as defined in SEQ ID NO:3; CDR-L1 having the sequence as defined in SEQ ID NO:4; CDR-L2 having the sequence as defined in SEQ ID NO:5 and CDR-L3 having the sequence as defined in SEQ ID NO:6; or
    b. comprises a light variable region having the sequence as defined in SEQ ID NO: 7 and a heavy variable region having the sequence as defined in SEQ ID NO: 8; or
    c. comprises a light variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 7 and a heavy variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 8;
    d. comprises a light variable region having the sequence as defined in SEQ ID NO: 7 and a heavy chain having the sequence as defined in SEQ ID NO: 11; or
    e. comprises a light variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 7 and a heavy chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 11; or
2) an antibody which comprises a light chain having the sequence as defined in SEQ ID NO: 9 and a heavy chain having the sequence as defined in SEQ ID NO: 10; or

3) an antibody which comprises a light chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 9 and a heavy chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 10.

Throughout this specification, complementarity determining regions ("CDR") are defined according to the Kabat definition. The Kabat definition is a standard for numbering the residues in an antibody and it is typically used to identify CDR regions (Kabat et al., (1991), 5th edition, NIH publication No. 91-3242).

Table 1

Region and SEQ ID identifier	**Amino acid sequence**
CDR-H1 SEQ ID NO:1	GFTFSNYGMV
CDR-H2 SEQ ID NO:2	YIDSDGDNTYYRDSVKG
CDR-H3 SEQ ID NO:3	GIVRPFLY
CDR-L1 SEQ ID NO:4	KSSQSLVGASGKTYLY
CDR-L2 SEQ ID NO:5	LVSTLDS
CDR-L3 SEQ ID NO:6	LQGTHFPHT
Light variable region SEQ ID NO:7	DIQMTQSPSS LSASVGDRVT ITCKSSQSLV GASGKTYLYW LFQKPGKAPK RLIYLVSTLD SGIPSRFSGS GSGTEFTLTI SSLQPEDFAT YYCLQGTHFP HTFGQGTKLE IK

Heavy variable region SEQ ID NO:8	EVPLVESGGG LVQPGGSLRL SCAVSGFTFS NYGMVWVRQA PGKGLEWVA IDSDGDNTYY RDSVKGRFTI SRDNAKSSLY LQMNSLRAED TAVYYCTTG VRPFLYWGQG TLVTVS
Light chain SEQ ID NO:9	DIQMTQSPSS LSASVGDRVT ITCKSSQSLV GASGKTYLYW LFQKPGKAPK RLIYLVSTLD SGIPSRFSGS GSGTEFTLTI SSLQPEDFAT YYCLQGTHFP HTFGQGTKLE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC
Heavy chain SEQ ID NO:10	EVPLVESGGG LVQPGGSLRL SCAVSGFTFS NYGMVWVRQA PGKGLEWVAY IDSDGDNTYY RDSVKGRFTI SRDNAKSSLY LQMNSLRAED TAVYYCTTGI VRPFLYWGQG TLVTVSSAST KGPSVFPLAP CSRSTSESTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPS SSLGTKTYTC NVDHKPSNTK VDKRVESKYG PPCPPCPAPE FLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSQEDPEV QFNWYVDGVE VHNAKTKPRE EQFNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKGLPSSIE KTISKAKGQP REPQVYTLPP SQEEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSRLTVD KSRWQEGNVF SCSVMHEALH NHYTQKSLSL SLGK
Fab heavy chain SEQ ID NO: 11	EVPLVESGGG LVQPGGSLRL SCAVSGFTFS NYGMVWVRQA PGKGLEWVA IDSDGDNTYY RDSVKGRFTI SRDNAKSSLY LQMNSLRAED TAVYYCTTG VRPFLYWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDY PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPS SSLGTQTYI NVNHKPSNTK VDKKVEPKSC

The antibody or antigen binding fragment thereof exemplified is further described in details in WO2014019727 (incorporated herein by reference).

Antibody molecules may be typically produced by culturing a host cell containing a vector encoding the antibody sequence under conditions suitable for leading to expression of protein from DNA encoding the antibody molecule of the present invention, and isolating the antibody molecule.

The antibody molecule may comprise only a heavy or light chain polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence needs to be used to transfect the host

cells. For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, a first vector encoding a light chain polypeptide and a second vector encoding a heavy chain polypeptide. Alternatively, a single vector may be used, the vector including sequences encoding light chain and heavy chain polypeptides.

An antibody or an antigen-binding fragment thereof that can be manufactured according to industrial scales can be produced by culturing eukaryotic host cells transfected with one or more expression vectors encoding the recombinant antibody fragment. The eukaryotic host cells are preferably mammalian cells, more preferably Chinese Hamster Ovary (CHO) cells.

Mammalian cells may be cultured in any medium that will support their growth and expression of the recombinant protein, preferably the medium is a chemically defined medium that is free of animal-derived products such as animal serum and peptone. There are different cell culture mediums available to the person skilled in the art comprising different combinations of vitamins, amino acids, hormones, growth factors, ions, buffers, nucleosides, glucose or an equivalent energy source, present at appropriate concentrations to enable cell growth and protein production. Additional cell culture media components may be included in the cell culture medium at appropriate concentrations at different times during a cell culture cycle that would be known to those skilled in the art.

Mammalian cell culture can take place in any suitable container such as a shake flask or a bioreactor, which may or may not be operated in a fed-batch mode depending on the scale of production required. These bioreactors may be either stirred-tank or air-lift reactors. Various large scale bioreactors are available with a capacity of more than 1,000 L to 50,000 L, preferably between 5,000 L and 20,000 L, or to 10,000 L. Alternatively, bioreactors of a smaller scale such as between 2 L and 100 L may also be used to manufacture an antibody or antibody fragment.

An antibody or antigen-binding fragment thereof is typically found in the supernatant of a mammalian host cell culture, typically a CHO cell culture. For CHO culture processes wherein the protein of interest such as an antibody or antigen-binding fragment thereof is secreted in the supernatant, said supernatant is collected by methods known in the art, typically by centrifugation.

Therefore, the antibody or antigen-binding fragment thereof production method comprises a step of centrifugation and supernatant recovery after cell culture and prior to protein purification. In a further particular embodiment said centrifugation is continuous centrifugation. For

avoidance of doubt, supernatant denotes the liquid lying above the sedimented cells resulting from the centrifugation of the cell culture.

Alternatively, host cells are prokaryotic cells, preferably gram-negative bacteria. More preferably, the host cells are *E. coli* cells. Prokaryotic host cells for protein expression are well known in the art (Terpe, K. Appl Microbiol Biotechnol 72, 211-222 (2006)). The host cells are recombinant cells which have been genetically engineered to produce the protein of interest such as an antigen-binding fragment of an antibody. The recombinant *E. coli* host cells may be derived from any suitable *E. coli* strain including from MC4100, TG1, TG2, DHB4, DH5α, DH1, BL21, K12, XL1Blue and JM109. One example is *E. coli* strain W3110 (ATCC 27,325) a commonly used host strain for recombinant protein fermentations. Antibody fragments can also be produced by culturing modified *E. coli* strains, for example metabolic mutants or protease deficient E. coli strains.

An antibody fragment is typically found in either the periplasm of the *E. coli* host cell or in the host cell culture supernatant, depending on the nature of the protein, the scale of production and the *E. coli* strain used. The methods for targeting proteins to these compartments are well known in the art (Makrides, S.C.; Microbiol Rev 60, 512-538 (1996)). Examples of suitable signal sequences to direct proteins to the periplasm of *E. coli* include the *E. coli* PhoA, OmpA, OmpT, LamB and OmpF signal sequences. Proteins may be targeted to the supernatant by relying on the natural secretory pathways or by the induction of limited leakage of the outer membrane to cause protein secretion examples of which are the use of the pelB leader, the protein A leader, the co-expression of bacteriocin release protein, the mitomycin-induced bacteriocin release protein along with the addition of glycine to the culture medium and the co-expression of the kil gene for membrane permeabilization. Most preferably, the recombinant protein is expressed in the periplasm of the host *E. coli*.

Expression of the recombinant protein in the *E. coli* host cells may also be under the control of an inducible system, whereby the expression of the recombinant antibody in *E. coli* is under the control of an inducible promoter. Many inducible promoters suitable for use in *E. coli* are well known in the art and depending on the promoter expression of the recombinant protein can be induced by varying factors such as temperature or the concentration of a particular substance in the growth medium. Examples of inducible promoters include the *E.coli* lac, tac, and trc promoters which are inducible with lactose or the non-hydrolyzable lactose analog, isopropyl-b-D-1-thiogalactopyranoside (IPTG) and the phoA, trp and araBAD promoters which are induced by phosphate, tryptophan and L-arabinose respectively. Expression may be induced by, for

example, the addition of an inducer or a change in temperature where induction is temperature dependent. Where induction of recombinant protein expression is achieved by the addition of an inducer to the culture the inducer may be added by any suitable method depending on the fermentation system and the inducer, for example, by single or multiple shot additions or by a gradual addition of inducer through a feed. It will be appreciated that there may be a delay between the addition of the inducer and the actual induction of protein expression for example where the inducer is lactose there may be a delay before induction of protein expression occurs while any pre-existing carbon source is utilized before lactose.

E. coli host cell cultures (fermentations) may be cultured in any medium that will support the growth of *E. coli* and expression of the recombinant protein. The medium may be any chemically defined medium such as e.g. described in Durany O, et al. (2004). Studies on the expression of recombinant fuculose-1-phosphate aldolase in Escherichia coli. Process Biochem 39, 1677-1684.

Culturing of the *E. coli* host cells can take place in any suitable container such as a shake flask or a fermenter depending on the scale of production required. Various large scale fermenters are available with a capacity of more than 1,000 liters up to about 100,000 liters. Preferably, fermenters of 1,000 to 50,000 liters are used, more preferably 1,000 to 25,000, 20,000, 15,000, 12,000 or 10,000 liters. Smaller scale fermenters may also be used with a capacity of between 0.5 and 1,000 liters.

Fermentation of *E. coli* may be performed in any suitable system, for example continuous, batch or fed-batch mode depending on the protein and the yields required. Batch mode may be used with shot additions of nutrients or inducers where required. Alternatively, a fed-batch culture may be used and the cultures grown in batch mode pre-induction at the maximum specific growth rate that can be sustained using the nutrients initially present in the fermenter and one or more nutrient feed regimes used to control the growth rate until fermentation is complete. Fed-batch mode may also be used pre-induction to control the metabolism of the *E. coli* host cells and to allow higher cell densities to be reached.

If desired, the host cells may be subject to collection from the fermentation medium, e.g. host cells may be collected from the sample by centrifugation, filtration or by concentration. In this case the process typically comprises a step of centrifugation and cell recovery prior to extracting the protein.

For *E. coli* fermentation processes wherein the protein of interest such as an antibody or antigen-binding fragment of an antibody is found in the periplasmic space of the host cell it is required to release the protein from the host cell. The release may be achieved by any suitable method such as cell lysis by mechanical or pressure treatment, freeze-thaw treatment, osmotic shock, extraction agents or heat treatment. Such extraction methods for protein release are well known in the art. Therefore in a particular embodiment, the production process comprises an additional protein extraction step prior to protein purification.

Other methods for obtaining antigen-binding fragment of a human antibody *in vitro* are based on display technologies such as phage display or ribosome display technology, wherein recombinant DNA libraries are used that are either generated at least in part artificially or from immunoglobulin variable (V) domain gene repertoires of donors. Phage and ribosome display technologies for generating human antibodies are well known in the art. Human antibodies may also be generated from isolated human B cells that are *ex vivo* immunized with an antigen of interest and subsequently fused to generate hybridomas which can then be screened for the optimal human antibody.

The pharmaceutical composition according to the invention may have a pH of 4.0 to 7.5, preferably of 4.0 to 6.0, more preferably 4.5 to 6.0, even more preferably from 5.5 to 5.8 or most preferred about 5.6.

The pharmaceutical composition contains a buffering agent to maintain the pH constant. There are many buffering agents used in the field of liquid pharmaceutical formulations, such as, but not limited to, citrate, phosphate, lactate, histidine, glutamate, maleate, tartrate, or succinate. A preferred buffer species is typically selected amongst those having a pKa that is close (+/-1 pH unit) to the preferred pH for optimal protein stability in order to maintain high buffering capacity, and is associated with the maximal demonstrated stability observed for a particular protein when placed in a series of varied buffer species. The adequate pH ranges of a formulation are generally chosen from those associated with the maximal demonstrated stability observed for a particular protein when placed in a series of varied pH formulations.

The pharmaceutical composition according to the invention comprises as a buffering agent, an amino acid or a mixture of amino acids, preferably at a concentration of 10mM to 100mM, 10mM to 80mM, 10mM to 60mM, 25mM to 60mM, preferably 30mM to 50mM, or 30mM.

Suitable amino acids are arginine, lysine, histidine, methionine, ornithine, isoleucine, leucine, alanine, glycine, glutamic acid or aspartic acid. The inclusion of a basic amino acid is preferred i.e. arginine, lysine and/or histidine. An amino acid may be present in its D- and/or L- form, but the L-form is typical. The amino acid may be present as any suitable salt e.g. a hydrochloride salt, such as arginine-HCl.

Preferably the amino acid is histidine, more preferably at a concentration of 30mM.

The pharmaceutical composition according to the invention may also comprise a mixture of amino acids for example a mixture of histidine and proline, histidine, proline and glycine, histidine, proline and arginine, histidine, proline, arginine and glycine. Preferably, the pharmaceutical composition according to the invention comprises histidine and another amino acid, preferably proline at a concentration of 10mM to 300mM, 50mM to 280mM, 100mM to 280mM or 180 mM to 250mM. Preferably, the pharmaceutical composition according to the invention comprises 30 mM histidine and from 180 mM to 250mM proline.

The pharmaceutical composition according to the invention additionally comprises a surfactant. Surfactants available for use in the pharmaceutical composition according to invention include, but are not limited to, non-ionic surfactants, ionic surfactants and zwitterionic surfactants. Typical surfactants for use with the invention include, but are not limited to, sorbitan fatty acid esters (e.g. sorbitan monocaprylate, sorbitan monolaurate, sorbitan monopalmitate), sorbitan trioleate, glycerine fatty acid esters (e.g. glycerine monocaprylate, glycerine monomyristate, glycerine monostearate), polyglycerine fatty acid esters (e.g. decaglyceryl monostearate, decaglyceryl distearate, decaglyceryl monolinoleate), polyoxyethylene sorbitan fatty acid esters (e.g. polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monostearate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene sorbitan trioleate, polyoxyethylene sorbitan tristearate), polyoxyethylene sorbitol fatty acid esters (e.g. polyoxyethylene sorbitol tetrastearate, polyoxyethylene sorbitol tetraoleate), polyoxyethylene glycerine fatty acid esters (e.g. polyoxyethylene glyceryl monostearate), polyethylene glycol fatty acid esters (e.g. polyethylene glycol distearate), polyoxyethylene alkyl ethers (e.g. polyoxyethylene lauryl ether), polyoxyethylene polyoxypropylene alkyl ethers (e.g. polyoxyethylene polyoxypropylene glycol, polyoxyethylene polyoxypropylene propyl ether, polyoxyethylene polyoxypropylene cetyl ether), polyoxyethylene alkylphenyl ethers (e.g. polyoxyethylene nonylphenyl ether), polyoxyethylene hydrogenated

castor oils {e.g. polyoxyethylene castor oil, polyoxyethylene hydrogenated castor oil), polyoxyethylene beeswax derivatives (e.g. polyoxyethylene sorbitol beeswax), polyoxyethylene lanolin derivatives (e.g. polyoxyethylene lanolin), and polyoxyethylene fatty acid amides (e.g. polyoxyethylene stearic acid amide); C_{10}-C_{18} alkyl sulfates (e.g. sodium cetyl sulfate, sodium lauryl sulfate, sodium oleyl sulfate), polyoxyethylene C_{10}-C_{18} alkyl ether sulfate with an average of 2 to 4 moles of ethylene oxide units added (e.g. sodium polyoxyethylene lauryl sulfate), and C_1-C_{18} alkyl sulfosuccinate ester salts (e.g. sodium lauryl sulfosuccinate ester); and natural surfactants such as lecithin, glycerophospholipid, sphingophospholipids (e.g. sphingomyelin), and sucrose esters of C_{12}-C_{18} fatty acids. The composition may include one or more of these surfactants. Preferred surfactants are polyoxyethylene sorbitan fatty acid esters e.g. polysorbate 20, 40, 60 or 80. Preferably the pharmaceutical compostion according to the invention comprises polysorbate 80.

The pharmaceutical composition of the invention may comprise from 0.01% to 10% w/v of surfactant, from 0.01% to 1.0% w/v of surfactant, from 0.01% to 0.5% w/v of surfactant or 0.03% w/v of surfactant such as polysorbates. Preferably, the pharmaceutical composition of the invention comprises 0.03% w/v of polysorbate 80.

The pharmaceutical composition according to the present invention comprises as a stabilizer (i.e. cryoprotectant) from 1 to 20% w/v, or from 5% to 13%w/v or from 7% to 10% w/v or 7% of sucrose.

The present invention also provides for a method for producing a pharmaceutical composition comprising an anti-FcRn antibody or antigen-binding fragment thereof, whereby the method comprises preparing a composition comprising an anti-FcRn antibody, for example a composition comprising from 80mg/mL to 140mg/mL of an anti-FcRn antibody or antigen-binding fragment thereof comprising a light variable chain comprising SEQ ID NO: 7 and a heavy variable chain comprising SEQ ID NO:8, 30 mM histidine, 250mM proline, 0.03% w/v polysorbate 80, at pH 5.6, and adding 1% to 25% w/v of sucrose.

Preferably, the method comprises preparing a composition comprising from 80mg/mL to 140mg/mL of an anti-FcRn antibody comprising a light variable chain comprising SEQ ID NO: 7 and a heavy variable chain comprising SEQ ID NO:8, 30 mM histidine, 250mM proline, 0.03%

w/v polysorbate 80, at pH 5.6, and adding a composition comprising 30 mM histidine, 0.03% w/v polysorbate 80, from 17.5% to 24.5% w/v of sucrose at pH 5.6.

The pharmaceutical compositions described and embodied herein may be in the form of a freeze-dried (i.e. lyophilized) pharmaceutical composition.

Hence, the method for producing a pharmaceutical composition according to the invention may further comprise the step of freeze-drying (i.e. lyophilizing).

Techniques for lyophilization of antibodies are well known in the art (John F. Carpenter and Michael J. Pikal, 1997 Pharm. Res. 14, 969-975); similarly there are available our days monoclonal antibody products such as SYNAGIS™, REMICADE™, RAPTIVA™, SIMULECT™, XOLAIR™ and HERCEPTIN™ which are supplied as lyophilizates. These antibodies are reconstituted to various final concentrations e.g. SIMULECT™ is reconstituted to a concentration of 4 mg/ml antibody, REMICADE™ is reconstituted to a concentration of 10 mg/ml, HERCEPTIN™ to 21 mg/ml, SYNAGIS™ and RAPTIVA™ to 100 mg/ml, and XOLAIR™ to 125 mg/ml.

Freeze-drying techniques will be discussed further herein the example section.

The present invention, therefore, provides for a freeze-dried formulation comprising an antibody or antigen-binding fragment thereof, sucrose, an amino acid or a mixture of amino acids and optionally a surfactant. Preferably the freeze-dried formulation comprises from 10% to 40% w/w sucrose, more preferably from 20% to 40% w/w sucrose.

In a preferred embodiment of the present invention the freeze-dried formulation comprising 10% to 40% w/w of sucrose also comprises an anti-FcRn antibody or antigen-binding fragment thereof wherein the antibody or fragment thereof comprises a light variable chain comprising SEQ ID NO:7 and a heavy variable chain comprising SEQ ID NO:8.

In another preferred embodiment, the freeze-dried formulation comprises about 50.8% w/w of antibody or antigen-binding fragment thereof, about 35.5% w/w/ sucrose, about 3.0% w/w histidine, about 10.5% w/w proline and 0.2% w/w polysorbate 80; wherein the antibody or fragment thereof comprises a light variable chain comprising SEQ ID NO:7 and a heavy variable chain comprising SEQ ID NO:8.

Additional excipients for use within the pharmaceutical compositions according to the invention include, but are not limited to, viscosity enhancing agents, bulking agents, solubilising agents

such as monosaccharides, e.g., fructose, maltose, galactose, glucose, D-mannose, sorbose and the like; disaccharides, e.g. lactose, trehalose, cellobiose, and the like; polysaccharides, e.g. raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and polyols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol) and the like; poly-ethylene glycols (e.g. PEG100, PEG300, PEG600, PEG1500, PEG2000, PEG3000, PEG3350, PEG4000, PEG6000, PEG8000 or PEG20000), polyvinylpyrrolidone, trimethylamine N-oxide, trimethylglycine or combinations thereof.

Before a freeze-dried (i.e. lyophilized) pharmaceutical composition can be administered to a patient it needs to be reconstituted with a solvent. Within the present invention, the preferred solvent is an aqueous solvent, more preferably the aqueous solvent is water.

The volume of solvent used for reconstitution dictates the concentration of the antibody or antigen-binding fragment thereof in the resulting liquid pharmaceutical formulation. Reconstitution with a smaller volume of solvent than the pre-lyophilization volume provides a formulation which is more concentrated than before lyophilization and vice-versa.

The present invention therefore provides for a method for reconstituting a freeze-dried pharmaceutical composition according to the invention by adding a solvent, wherein the solvent is preferably water.

The reconstitution ratio (volume of pre-lyophilized pharmaceutical composition to solvent used to reconstitute the freeze-dried pharmaceutical composition) may vary from 0.5:1 to 1:10. In a preferred embodiment, a ratio of about 1:1 is applied so that the resulting volume of the reconstituted liquid pharmaceutical formulation is about 4.4mL.

Preferred pharmaceutical compositions pre-lyophilization and/or reconstituted liquid pharmaceutical formulations according to the invention comprise:

A. from 1mg/mL to 200mg/mL of an antibody or antigen-binding fragment thereof comprising a light variable chain comprising SEQ ID NO: 7 and a heavy variable chain comprising SEQ ID NO:8, from 10 mM to 50mM histidine, from 10 to 300mM proline, from 1% to 20% w/v sucrose, from 0.01% to 1.0% w/v polysorbate 80, at pH 5.6;
B. from 50mg/mL to 150mg/mL of an antibody or antigen-binding fragment thereof comprising a light variable chain comprising SEQ ID NO: 7 and a heavy variable

chain comprising SEQ ID NO:8, from 20 mM to 40mM histidine, from 50 to 280mM proline, from 5% to 13% w/v sucrose, from 0.01% to 0.5% w/v polysorbate 80, at pH 5.6;

C. from 80mg/mL to 140mg/mL of an antibody or antigen-binding fragment thereof comprising a light variable chain comprising SEQ ID NO: 7 and a heavy variable chain comprising SEQ ID NO:8, 30 mM histidine, 250mM proline, 7% w/v sucrose, 0.03% w/v polysorbate 80, at pH 5.6;

D. from 50mg/mL to 150mg/mL of an antibody or antigen-binding fragment thereof comprising a light variable chain comprising SEQ ID NO: 7 and a heavy variable chain comprising SEQ ID NO:8 or a heavy chain comprising SEQ ID NO:11, 30 mM histidine, 180mM proline, 7% w/v sucrose, 0.03% w/v polysorbate 80, at pH 5.6;

E. from 80mg/mL to 140mg/mL, preferably 100mg/ml, of antibody or antigen-binding fragment thereofcomprising a heavy variable chain and a light variable chain, wherein the heavy variable chain comprises CDR-H1 of SEQ ID NO:1, CDR-H2 of SEQ ID NO:2 and CDR-H3 of SEQ ID NO:3 and the light variable chain comprises CDR-L1 of SEQ ID NO:4, CDR-L2 of SEQ ID NO:5 and CDR-L3 of SEQ ID NO:6, 30 mM histidine, 180mM proline, 7% w/v sucrose, 0.03% w/v polysorbate 80, at pH 5.6;

F. from 80mg/mL to 140mg/mL, preferably 100mg/ml, of antibody comprising a heavy chain comprising SEQ ID NO: 10 and a light chain comprising SEQ ID NO:9, 30 mM histidine, 180mM proline, 7% w/v sucrose, 0.03% w/v polysorbate 80, at pH 5.6 and at least i) or i) and ii) or i), ii) and iii):
   i) from 100mM to 250mM of mannitol;
   ii) from 80mM to 200mM of L-arginine HCl;
   iii) from 100mM to 280mM of glycine;

G. from 80mg/mL to 140mg/mL, preferably 100mg/ml, of an antibody or antigen-binding fragment thereof comprising a light variable chain comprising SEQ ID NO: 7 and a heavy variable chain comprising SEQ ID NO:8 or a heavy chain comprising SEQ ID NO:11, 30 mM histidine, 180mM proline, 7% w/v sucrose, 0.03% w/v polysorbate 80, at pH 5.6 and at least i) or i) and ii) or i), ii) and iii):
   i) from 100mM to 250mM of mannitol;
   ii) from 80mM to 200mM of L-arginine HCl;
   iii) from 100mM to 280mM of glycine;

H. from 80mg/mL to 140mg/mL, preferably 100mg/ml, of an antibody or antigen-binding fragment thereof comprising a heavy variable chain and a light variable chain,

wherein the heavy variable chain comprises CDR-H1 of SEQ ID NO:1, CDR-H2 of SEQ ID NO:2 and CDR-H3 of SEQ ID NO:3 and the light variable chain comprises CDR-L1 of SEQ ID NO:4, CDR-L2 of SEQ ID NO:5 and CDR-L3 of SEQ ID NO:6, 30 mM histidine, 180mM proline, 7% w/v sucrose, 0.03% w/v polysorbate 80, at pH 5.6 and at least i) or i) and ii) or i), ii) and iii):

i) from 100mM to 250mM of mannitol;

ii) from 80mM to 200mM of L-arginine HCl;

iii) from 100mM to 280mM of glycine;

l. from 80mg/mL to 140mg/mL, preferably 100mg/ml, of an antibody comprising a heavy chain comprising SEQ ID NO: 10 and a light chain comprising SEQ ID NO:9, 30 mM histidine, 180mM proline, 7% w/v sucrose, 0.03% w/v polysorbate 80, at pH 5.6 and at least i) or i) and ii) or i), ii) and iii):

i) from 100mM to 250mM of mannitol;

ii) from 80mM to 200mM of L-arginine HCl;

iii) from 100mM to 280mM of glycine;

The present invention also provides for a container comprising the freeze-dried pharmaceutical composition or the reconstituted liquid pharmaceutical formulation according to the invention. In particular, the container may be a vial comprising the freeze-dried pharmaceutical composition to be reconstituted.

The container may be part of a kit-of-parts comprising one or more containers comprising the freeze-dried pharmaceutical compositions according to the invention, suitable solvents for reconstituting the freeze-dried pharmaceutical composition, delivery devices such as a syringes and instructions of use.

The pharmaceutical compositions or the liquid pharmaceutical formulations according to the invention are for use in therapy.

In a preferred embodiment, the reconstituted liquid pharmaceutical formulation for use in therapy comprises from 80mg/mL to 140mg/mL, preferably 100mg/ml, of an antibody or antigen-binding fragment thereof, 30 mM histidine, 180mM proline, 7% w/v sucrose, 0.03% w/v polysorbate 80, at pH 5.6 optionally, i) or i) and ii) or i), ii) and iii) wherein:

i) from 100mM to 250mM of mannitol;

ii) from 80mM to 200mM of L-arginine HCl;

iii) from 100mM to 280mM of glycine;

wherein the antibody or antigen-binding fragment thereof (as it may apply) comprises:

1) a light variable chain comprising SEQ ID NO: 7 and a heavy variable chain comprising SEQ ID NO:8 or a heavy chain comprising SEQ ID NO:11; or
2) a light variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 7 and a heavy variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 8;
3) a light variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 7 and a heavy chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 11;
4) a light chain comprising SEQ ID NO: 9 and a heavy chain comprising SEQ ID NO:10; or
5) a light chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 9 and a heavy chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 10;
6) a heavy variable chain and a light variable chain, wherein the heavy variable chain comprises CDR-H1 of SEQ ID NO:1, CDR-H2 of SEQ ID NO:2 and CDR-H3 of SEQ ID NO:3 and the light variable chain comprises CDR-L1 of SEQ ID NO:4, CDR-L2 of SEQ ID NO:5 and CDR-L3 of SEQ ID NO:6.

The pharmaceutical composition or the liquid pharmaceutical formulations according to the invention are also for use in the treatment of inflammatory or autoimmune diseases selected from myasthenia gravis, Pemphigus vulgaris, Neuromyelitis optica, Guillain-Barré syndrome, lupus, and thrombotic thrombocytopenic purpura, Idiopathic thrombocytopenic purpura (ITP), Chronic inflammatory demyelinating polyneuropathy (CIDP) and combinations thereof.

In another preferred embodiment, the reconstituted liquid pharmaceutical formulation for use in the treatment of inflammatory or autoimmune diseases selected from myasthenia gravis, Pemphigus vulgaris, Neuromyelitis optica, Guillain-Barré syndrome, lupus, and thrombotic thrombocytopenic purpura, Idiopathic thrombocytopenic purpura (ITP), Chronic inflammatory demyelinating polyneuropathy (CIDP) and combinations thereof comprises from 80mg/mL to

140mg/mL, preferably 100mg/ml, of an antibody or antigen-binding fragment thereof, 30 mM histidine, 180mM proline, 7% w/v sucrose, 0.03% w/v polysorbate 80, at pH 5.6, optionally, i) or i) and ii) or i), ii) and iii) wherein:

i) from 100mM to 250mM of mannitol;

ii) from 80mM to 200mM of L-arginine HCl;

iii) from 100mM to 280mM of glycine;

wherein the antibody or antigen-binding fragment thereof (as it may apply) comprises:

1) a light variable chain comprising SEQ ID NO: 7 and a heavy variable chain comprising SEQ ID NO:8 or a heavy chain comprising SEQ ID NO:11; or

2) a light variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 7 and a heavy variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 8;

3) a light variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 7 and a heavy chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 11;

4) a light chain comprising SEQ ID NO: 9 and a heavy chain comprising SEQ ID NO:10; or

5) a light chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 9 and a heavy chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 10;

6) a heavy variable chain and a light variable chain, wherein the heavy variable chain comprises CDR-H1 of SEQ ID NO:1, CDR-H2 of SEQ ID NO:2 and CDR-H3 of SEQ ID NO:3 and the light variable chain comprises CDR-L1 of SEQ ID NO:4, CDR-L2 of SEQ ID NO:5 and CDR-L3 of SEQ ID NO:6.

The invention also provides for a method for treating an inflammatory or autoimmune disease in a mammalian subject comprising administering the pharmaceutical composition or the liquid pharmaceutical formulations according to the invention; wherein the inflammatory or autoimmune disease is selected from myasthenia gravis, Pemphigus vulgaris, Neuromyelitis

optica, Guillain-Barré syndrome, lupus, and thrombotic thrombocytopenic purpura, Idiopathic thrombocytopenic purpura (ITP), Chronic inflammatory demyelinating polyneuropathy (CIDP) and combinations thereof.

The mammalian subject may be selected from the group of rodents, cats, dogs, horses, bovine, ovine, non-human primates and human subjects. Preferably, the mammalian subject is a human subject.

In yet another preferred embodiment the method for treating an inflammatory or autoimmune disease in a mammalian subject comprising administering the reconstituted liquid pharmaceutical formulation comprising from 80mg/mL to 140mg/mL, preferably 100mg/ml, of an antibody or antigen-binding fragment thereof, 30 mM histidine, 180mM proline, 7% w/v sucrose, 0.03% w/v polysorbate 80, at pH 5.6, optionally, i) or i) and ii) or i), ii) and iii) wherein:

iv) from 100mM to 250mM of mannitol;

i) from 80mM to 200mM of L-arginine HCl;

ii) from 100mM to 280mM of glycine;

wherein the antibody or antigen-binding fragment thereof (as it may apply) comprises:

1) a light variable chain comprising SEQ ID NO: 7 and a heavy variable chain comprising SEQ ID NO:8 or a heavy chain comprising SEQ ID NO:11; or

2) a light variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 7 and a heavy variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 8;

3) a light variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 7 and a heavy chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 11;

4) a light chain comprising SEQ ID NO: 9 and a heavy chain comprising SEQ ID NO:10; or

5) a light chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 9 and a heavy chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 10;

6) a heavy variable chain and a light variable chain, wherein the heavy variable chain comprises CDR-H1 of SEQ ID NO:1, CDR-H2 of SEQ ID NO:2 and CDR-H3 of SEQ ID NO:3 and the light variable chain comprises CDR-L1 of SEQ ID NO:4, CDR-L2 of SEQ ID NO:5 and CDR-L3 of SEQ ID NO:6.

and wherein the inflammatory or autoimmune disease is selected from myasthenia gravis, Pemphigus vulgaris, Neuromyelitis optica, Guillain-Barré syndrome, lupus, and thrombotic thrombocytopenic purpura, Idiopathic thrombocytopenic purpura (ITP), Chronic inflammatory demyelinating polyneuropathy (CIDP) and combinations thereof.

Furthermore, the pharmaceutical composition or the liquid pharmaceutical formulations according to the invention may be used in the manufacture of a medicament for the treatment of inflammatory or autoimmune diseases selected from myasthenia gravis, Pemphigus vulgaris, Neuromyelitis optica, Guillain-Barré syndrome, lupus, and thrombotic thrombocytopenic purpura, Idiopathic thrombocytopenic purpura (ITP), Chronic inflammatory demyelinating polyneuropathy (CIDP) and combinations thereof.

In another preferred embodiment, the reconstituted liquid pharmaceutical formulation may be used in the manufacture of a medicament for the treatment of inflammatory or autoimmune diseases selected from myasthenia gravis, Pemphigus vulgaris, Neuromyelitis optica, Guillain-Barré syndrome, lupus, and thrombotic thrombocytopenic purpura, Idiopathic thrombocytopenic purpura (ITP), Chronic inflammatory demyelinating polyneuropathy (CIDP) and combinations thereof comprises from 80mg/mL to 140mg/mL, preferably 100mg/ml, of an antibody or antigen-binding fragment thereof, 30 mM histidine, 180mM proline, 7% w/v sucrose, 0.03% w/v polysorbate 80, at pH 5.6, optionally, i) or i) and ii) or i), ii) and iii) wherein:

v) from 100mM to 250mM of mannitol;

vi) from 80mM to 200mM of L-arginine HCl;

vii) from 100mM to 280mM of glycine;

wherein the antibody or antigen-binding fragment thereof (as it may apply) comprises:

1) a light variable chain comprising SEQ ID NO: 7 and a heavy variable chain comprising SEQ ID NO:8 or a heavy chain comprising SEQ ID NO:11; or

2) a light variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 7 and a heavy variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 8;

3) a light variable region having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 7 and a heavy chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 11;

4) a light chain comprising SEQ ID NO: 9 and a heavy chain comprising SEQ ID NO:10; or

5) a light chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 9 and a heavy chain having at least 80% identity or similarity, preferably 90% identity or similarity to the sequence as defined in SEQ ID NO: 10;

6) a heavy variable chain and a light variable chain, wherein the heavy variable chain comprises CDR-H1 of SEQ ID NO:1, CDR-H2 of SEQ ID NO:2 and CDR-H3 of SEQ ID NO:3 and the light variable chain comprises CDR-L1 of SEQ ID NO:4, CDR-L2 of SEQ ID NO:5 and CDR-L3 of SEQ ID NO:6.

The reconstituted liquid pharmaceutical formulations according to the invention are administered in a therapeutically effective amount. The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent (i.e. an antibody) needed to treat, ameliorate or prevent a targeted disease, disorder or condition, or to exhibit a detectable therapeutic, pharmacological or preventative effect. For any antibody or antigen-binding fragments thereof, the therapeutically effective amount can be estimated initially either in cell culture assays or in animal models, usually in rodents, rabbits, dogs, pigs or primates. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

The precise therapeutically effective amount for a human subject will depend upon the severity of the disease state, the general health of the subject, the age, weight and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities and tolerance/response to therapy. This amount can be determined by routine experimentation and is within the judgement of the clinician. Generally, a therapeutically effective amount of antibody will be from 0.01 mg/kg to 500 mg/kg, for example 0.1 mg/kg to 200 mg/kg, such as 100 mg/kg.

For the treatment of the above diseases and/or disorders, the appropriate dosage will vary depending upon, for example, the particular antibody to be employed, the subject treated, the mode of administration and the nature and severity of the condition being treated. In a particular embodiment, the reconstituted liquid pharmaceutical formulation of the invention is administered by intravenous or subcutaneous route. When administered via intravenous injection, it may be administered as a bolus injection or as a continuous infusion. The reconstituted liquid pharmaceutical formulation according to any of the embodiments of the invention may also be administered by intramuscular injection. The reconstituted liquid pharmaceutical formulation may be injected using a syringe, an injection device such as an autoinjector, a needleless device, an implant and a patch.

In one specific embodiment of the present invention, a dosage of 400mg is achieved by administering 4mL of the 1:1 reconstituted liquid pharmaceutical formulation comprising 100mg/mL of anti-FcRn antibody of antigen-binding fragment thereof.

The liquid pharmaceutical formulation of the invention is suitably administered to the patient at one time or over a series of treatments and may be administered to the patient at any time from diagnosis onwards; it may be administered as the sole treatment or in conjunction with other drugs or therapies useful in treating the conditions as described herein before.

The antibody or antigen-binding fragment thereof may be the sole active ingredient in the liquid pharmaceutical formulation. Alternatively, the antibody or antigen-binding fragment thereof may be administered in combination, e.g. simultaneously, sequentially or separately, with one or more other therapeutically active ingredients. Active ingredient as employed herein refers to an ingredient with a pharmacological effect, such as a therapeutic effect, at a relevant dose. In some embodiments the antibody or antigen-binding fragment thereof in the liquid pharmaceutical formulation may be accompanied by other active ingredients including other antibodies or non-antibody ingredients. When the antibody in the liquid pharmaceutical formulation according to the invention is an anti-FcRn antibody, the subject may be administered with an additional active ingredient to treat inflammatory or autoimmune disease. In one embodiment, the subject is administered, simultaneously or in sequence (before and/or after) other antibody ingredients or non-antibody ingredients such as steroids or other drug molecules, in particular drug molecules whose half-life is independent of FcRn binding.

The invention will now be further described by way of examples with references to embodiments illustrated in the accompanying drawings

EXAMPLES

Abbreviations

DLS (Dynamic Light Scattering); Arg (L-arginine); iCE (Imaged capillary electrophoresis); Mal (Maltitol); Pro (L-Proline); PS80 (Polysorbate 80); Raf (Raffinose); SE-UPLC (Size-exclusion Ultra Performance Liquid Chromatography); Sor (Sorbitol); Suc (Sucrose); Gly (Glycine); Man (Mannitol); Met (L-methionine); Tre (Trehalose).

Materials

D-(-)-Sorbitol (VWR GPR Rectopur™); Polysorbate 80 (Tween 80™, Merck™); 140mg/mL of an anti-FcRn antibody as described in WO2014019727 (SEQ ID NOs: 9 and 10) in 30mM histidine, 250mM proline, 0.03% PS80, pH5.6 (UCB Celltech ltd); Mannitol (Roquette™); all the following from (Sigma™): D-(+)-Raffinose pentahydrate, L-Arginine monohydrochloride, L-Histidine, L-Proline, Maltitol, Glycine, Sucrose, L-methionine and Trehalose dehydrate.

Example 1

Freeze-dried formulation preparation

Freeze-drying formulations comprising 100mg/mL and 80mg/mL of an anti-FcRn antibody as described in WO2014019727 (SEQ ID NOs: 9 and 10) were prepared by spiking a composition comprising 140mg/mL of said antibody in 30mM histidine, pH5.6, 250mM proline, 0.03% PS80 (reference formulation) with the solutions A-I as indicated in Table 2 below.

Table 2

Ref.	Conc (mg/mL)	Formulation	Conc (mg/mL)	Formulation	
	Initial				

Ref.	Conc (mg/mL)	Formulation	Conc (mg/mL)	Formulation	
	Spiking (100:40) with		**Final**		
A	N/A	30mM histidine pH5.6, 0.03% PS80, 17.5% sucrose	100	30mM histidine pH5.6, 180mM proline, 0.03% PS80, 5% sucrose	100-Suc5
B		30mM histidine pH5.6, 0.03% PS80, 24.5% sucrose		30mM histidine pH5.6, 180mM proline, 0.03% PS80, 7% sucrose	100-Suc7
C		30mM histidine pH5.6, 0.03% PS80, 14% sorbitol		30mM histidine pH5.6, 180mM proline, 0.03% PS80, 4% sorbitol	100-Sor4
D		30mM histidine pH5.6, 0.03% PS80, 24.5% maltitol		30mM histidine pH5.6, 180mM proline, 0.03% PS80, 7% maltitol	100-Mal7
E		30mM histidine pH5.6, 0.03% PS80, 35% raffinose		30mM histidine pH5.6, 180mM proline, 0.03% PS80, 10% raffinose	100-Raf10
F		30mM histidine pH5.6, 0.03% PS80, 630mM L-arginine		30mM histidine pH5.6, 180mM proline, 0.03% PS80, 180mM L-arginine	100-Arg180
G		30mM histidine pH5.6, 0.03% PS80, 250mM proline		30mM histidine pH5.6, 250mM proline**Fehler! Verweisquelle konnte nicht gefunden werden.**, 0.03% PS80	100-Pro250
	Spiking (80:60) with		**Final**		
H	N/A	30mM histidine pH5.6, 0.03% PS80, 16.5% sucrose	80	30mM histidine pH5.6, 180mM proline, 0.03% PS80, 7% sucrose	80-Suc7
I		30mM histidine pH5.6, 0.03% PS80, 21% sucrose		30mM histidine pH5.6, 180mM proline, 0.03% PS80, 9% sucrose	80-Suc9

About 3mL vials (Schott Topline Fiolax™ clear) were filled with 1mL of freeze-drying formulation. Freeze-drying was done with a Martin-Christ Epsilon 2-6D™ freeze-dryer. A lyophilization cycle consisted of freezing at -40°C, annealing at -10°C, sublimation at -20°C, and secondary drying at 25°C (Table 3). At the end of the lyophilization cycle vials were filled with approximately 400mbar N2 and closed with single-vent stoppers (Westar™, West

Pharmaceuticals™). Lyophilisates were stored at 2-8°C until analysis or the start of the stability studies.

Table 3

step	t (h)	ramp (°C/min)	hold (min)	T (°C)	p (mbar)
1	0.00		0	5	1000
2	1.50	0.50		-40	1000
3	2.50		60	-40	1000
4	3.50	0.50		-10	1000
5	5.50		120	-10	1000
6	6.50	0.50		-40	1000
7	8.50		120	-40	1000
8	9.00		30	-40	0.28
9	9.67	0.50		-20	0.28
10	33.67		1440	-20	0.28
11	35.17	0.50		25	0.28
12	41.17		360	25	0.28
13	41.50	1.00		5	0.28
14	50.00		0	5	0.28

The stability studies carried out in these experiments consisted of an accelerated stability study for 3 and 6 months at 30°C, and stressed stability studies for 4 weeks and 3 months at 40°C).

1. Reconstitution

Lyophilisates were reconstituted with 0.9mL Milli-Q water to a concentration of 80 or 100mg/mL respectively. The time for the cake to completely dissolve to a clear solution (with possibly a rim

of foam on top) was recorded. Appearance of the lyophilisate and the reconstituted solution was visually assessed.

The reconstitution times are shown in Figure 1. The reconstitution time is shown on the y-axis in minutes per each of the formulations as per Table 2. The measurements were performed on a formulation reconstituted just after lyophilisation (no storage; t0); after reconstitution of the lyophilised pharmaceutical composition after storage for 3 months at 30°C (t3m, 30°C); after 6 months at 30°C (t6m, 30°C); after 4 weeks at 40°C (t4w, 40°C) and after 3 months at 40°C (t3m, 40°C).

Reconstitution times were within 10 minutes for the formulations containing sucrose and for the formulation with 180mM L-arginine (Figure 1). Formulation comprising 80mg/mL of the anti-FcRn antibody reconstituted significantly faster than those formulations comprising 100mg/mL. Formulations comprising 100mg/mL of the anti-FcRn antibody in 7% sucrose or in 180mM L-arginine showed acceptable reconstitution times of less than 10min at all-time points of the stability studies.

Aggregation

The anti-FcRn antibody concentration was determined by UV absorbance at 280nm undiluted using a SoloVPE™ (CE Technologies™) extension connected to a Cary50™ UV-visible spectrophotometer (Varian™), or after dilution in water to 1mg/mL with a Spectramax™ M5 plate reader (Molecular Devices™), $\varepsilon = 1.34 mL.mg^{-1}.cm^{-1}$.

The quantity of protein aggregation was determined by size-exclusion UPLC (SE-UPLC) using two Acquity™ BEH200 SEC (Waters) 1.7µm, 4.6x150mm columns in series and 0.1M Na-phosphate, 0.3M NaCl, pH7.0 running buffer (0.3mL/min). Detection and quantitation were performed by fluorescence (ex 280nm, em 345nm).

Protein aggregation was also evaluated by dynamic light scattering (DLS) using a DynaPro Plate Reader™ (Wyatt™). Sample aliquots diluted to 10mg/mL in 30mM histidine pH 5.6 were measured (10 acquisitions of 5 sec). Data were analyzed with the Dynamics™ 7.1.4 regularization fit for multimodal particle distribution (Wyatt™).

Data are shown in Figure 2 as the total percentage of aggregates determined by SE UPLC per each formulation. The reference formulation (140mg/mL of the anti-FcRn antibody in 30mM histidine, pH5.6, 250mM proline, 0.03% PS80; not lyophilised) was also investigated (Ref in

Figure 2). Measurements were performed on a formulation reconstituted just after lyophilisation (no storage; t0); after reconstitution of the lyophilised pharmaceutical compositions after storage for 3 months at 30°C (t3m, 30°C); after 6 months at 30°C (t6m, 30°C); after 4 weeks at 40°C (t4w, 40°C) and after 3 months at 40°C (t3m, 40°C).

Except for the proline formulation (100-Pro250) all formulations showed an increased stability towards aggregation compared to the reference formulation (Figure 2). In particular formulations containing ≥7% sucrose showed good stability towards aggregation both as absolute aggregate formation per each stability study and as a rate of aggregate formation over studies conducted at 30°C and 40°C. A formulation comprising 100mg/mL of the anti-FcRn antibody in 30mM histidine pH5.6, 180mM proline, 7% sucrose, 0.03% PS80 showed an increase in aggregates of only 0.2%/month at 30°C and 0.4%/month at 40°C (Table 4).

Table 4

	aggr (% area)					**aggr rate (%area/month)**	
	t_0	**$t_{3m30°C}$**	**$t_{6m30°C}$**	**$t_{4w40°C}$**	**$t_{3m40°C}$**	**30°C**	**40°C**
Ref	3.7	7.0	7.6	8.0	13.5	0.9	3.8
100-Suc5	3.6	4.6	4.8	4.6	5.4	0.3	0.8
100-Suc7	3.6	4.3	4.4	4.2	4.8	**0.2**	**0.5**
100-Sor4	3.6	4.9	5.4	5.3	6.9	0.4	1.4
100-Mal7	3.6	4.2	4.2	4.1	4.7	0.1	0.5
100-Raf10	3.6	4.5	4.7	4.5	5.2	0.2	0.7
100-Arg180	3.5	4.6	4.9	4.6	5.6	0.3	0.9
100-Pro250	3.9	7.1	8.3	7.4	11.1	1.0	3.1
80-Suc7	3.5	4.0	4.0	3.9	4.3	0.1	0.3
80-Suc9	3.5	3.8	3.7	3.8	4.1	0.1	0.2

Maltitol showed also acceptable aggregate formation, in line with what observed with ≥7% sucrose. Whilst arginine and maltitol showed reasonable results in the reconstitution time and aggregate formation, respectively, they did not provide acceptable results in both tests, unlike

≥7% sucrose which provided low reconstitution time, low aggregate formation and rate of formation.

Aggregates not detected by SE UPLC were investigated by DLS. The percentage aggregates determined by DSL using the percentage intensity size distribution is shown per each of the lyophilised compositions, before lyophilisation and on formulations reconstituted just after lyophilisation (no storage; t0); after reconstitution of the lyophilised pharmaceutical compositions after storage for 3 months at 30°C (t3m, 30°C); after 6 months at 30°C (t6m, 30°C); after 4 weeks at 40°C (t4w, 40°C) and after 3 months at 40°C (t3m, 40°C).

The reference formulation (140mg/mL of the anti-FcRn antibody in 30mM histidine, pH5.6, 250mM proline, 0.03% PS80; not lyophilised) was also investigated. Neither the reference (Ref) nor the freeze-dried formulations showed a significant amount of HMW aggregates. No significant change in large or HMW aggregates upon stability was observed by DLS (Figure 3) as the measured variations in intensity are not significant and inherent to the measurement technique.

Charge variants

Charge variants were determined by imaged capillary electrophoresis (iCEwith iCE3 (Protein Simple™). Measurements were performed on formulations reconstituted just after lyophilisation (no storage; t0); after reconstitution of the lyophilised pharmaceutical compositions after storage for 3 months at 30°C (t3m, 30°C); after 6 months at 30°C (t6m, 30°C); after 4 weeks at 40°C (t4w, 40°C) and after 3 months at 40°C (t3m, 40°C).

An increase in acidic species (Figure 4A) was observed for the reference formulation (140mg/mL of the anti-FcRn antibody in 30mM histidine, pH5.6, 250mM proline, 0.03% PS80; not lyophilised). The formulation containing 250mM proline showed a decrease in acidic species but it also showed a significant increase in basic species (Figure 4B). The formulation comprising 4% sorbitol also showed a marked increase in basic species. The remaining freeze-dying formulations did show only a minor increase in basic species and no significant change in acidic species (Figure 4A ad 4B). The formulations comprising ≥7% sucrose did not show any significant charge variants changes.

Viscosity

Viscosity of reconstituted product was measured with a ViscoPro2000™ viscometer (Cambridge Viscosity). Measurements were performed on formulations reconstituted just after lyophilisation (no storage) at 22 +/-1°C.

Viscosity increased with increasing sugar/sugar alcohol concentration or with increasing sugar/sugar alcohol molecular weight (Figure 5). The viscosity for the formulation comprising 80 and 100mg/mL in sucrose freeze-drying formulation were all acceptable. The formulation comprising 100mg/mL of the anti-FcRn antibody in combination with 180mM L-arginine showed also acceptable viscosity. Arginine is a recognised excipient which is commonly added to pharmaceutical compositions to reduce the viscosity. Its effect was comparable to the formulation comprising sucrose.

Osmolality

Osmolality of reconstituted formulations was measured with a Vapro™ 5520 vapor pressure osmometer (Wescor™). Measurements were performed on formulations reconstituted just after lyophilisation (no storage).

Most formulations showed an osmolality of around 500mOsm/kg (Figure 6). The formulation comprising 100mg/mL of the anti-FcRn antibody in the 180mM L-arginine formulation showed the highest osmolality (570mOsm/kg), whilst formulation comprising 100mg/mL of the anti-FcRn antibody in the 250mM proline formulation showed the lowest osmolality (330mOsm/kg). The formulation comprising sucrose showed acceptable osmolality.

Example 2

Freeze-drying formulations (Table 5), except for the reference formulation, were prepared from 60mg/mL the anti-FcRn antibody in 30mM histidine, 250mM sorbitol, pH5.6 by buffer exchange using Amicon™ Ultra-15 30K (Millipore™) centrifugal filter/concentration devices for volumes up to 15mL with at least 4 cycles of dilution/concentration using in total a diavolume of at least 7 times the volume of the original sample to the final compositions according to Table 5 at pH5.6. Polysorbate 20 (PS20) was added after buffer exchange from a filtered 3% PS20 stock solution to a final concentration of 0.03%. Formulation B of Example 1 (100mg/ml of the anti-FcRn antibody, 7% sucrose, 30 mM histidine, 180 mM proline and 0.03% PS80, pH 5.6) was chosen as the reference formulation for the experiments of Example 2.

Vials (3mL; Topline Fiolac clear, Schott™) were filled with 1mL of formulation comprising 100mg/mL or 140mg/mL of the anti-FcRn antibody (indicated by * are formulations tested at both concentrations of 100mg/ml and 140mg/ml) (Table 5). Freeze-drying was performed as for Example 1.

Table 5

Formulation										
		% w/v	**mM**		**% w/v**	**mM**		**%w/v**		**mM**
Reference	Sucrose	7	204	L-proline		180	PS80	0.03	histidine	30
Suc7		7	204		N/A	N/A	PS20	0.03	histidine	30
Suc10*		10	292		N/A	N/A				
Suc13		13	380		N/A	N/A				
Suc5Man4		5	146	Mannitol	4	220				
Suc6Man3*		6	175		3	165				
Suc7Man2		7	204		2	110				
Suc5Arg4		5	146	L-arginineHCl	4	190				
Suc6Arg3*		6	175		3	142				
Suc7Arg2		7	204		2	95				
Suc5Gly2		5	146	Glycine	2	266				
Suc6Gly1.5*		6	175		1.5	200				
Suc7Gly1		7	204		1	133				
Suc5Met3		5	146	L-methionine	3	201				
Suc6Met2.25*		6	175		2.25	151				
Suc7Met1.5		7	204		1.5	100				
Tre10*	Trehalose (dihydrate)	10	264		N/A	N/A				

Reconstitution

Reconstitution was carried out as per Example 1. Measurements were performed on formulations reconstituted just after lyophilisation (no storage; t0); after reconstitution of the

lyophilised pharmaceutical compositions after storage for 3 months at 30°C (t3m, 30°C); after 6 months at 30°C (t6m, 30°C); after 4 weeks at 40°C (t4w, 40°C) and after 3 months at 40°C (t3m, 40°C). Relatively rapid reconstitution was achieved for sucrose, sucrose-glycine and sucrose-arginine formulations, both at 100 mg/mL and 140 mg/mL of the anti-FcRn antibody (Figures 7A, Figure 7B). Sucrose-mannitol formulations did not reconstitute comparably fast, whilst methionine formulations and the trehalose formulation required long time to reconstitute (see different y-axis scale in figure 7A). The difference between the 10% sucrose and the 10% trehalose formulation is surprising and suggests a specific interaction between sucrose and the anti-FcRn antibody aiding reconstitution. The negative influence of methionine on reconstitution could be due to change in the structure of the lyophilized cake, but also to molecular changes of the anti-FcRn antibody (more evident in the aggregation analysis below).

Aggregation

Aggregation was investigated according to the method of Example 1. Measurements were performed on formulations prior lyophilisation, on formulations reconstituted just after lyophilisation (no storage; t0); after reconstitution of the lyophilised pharmaceutical compositions after storage for 3 months at 30°C (t3m, 30°C); after 6 months at 30°C (t6m, 30°C); after 4 weeks at 40°C (t4w, 40°C) and after 3 months at 40°C (t3m, 40°C). All formulations, except for the methionine containing formulation, at both concentrations (100mg/ml and 140mg/mL) of the anti-FcRn antibody, showed good stability towards aggregation (Figures 8A and 8B). Formulations showing the highest stability towards aggregation included the 10% and 13% sucrose formulations, the 6% sucrose-3% mannitol and 7% sucrose-2% mannitol formulations, all arginine containing formulations, and the reference formulation (containing 7% sucrose and proline). Aggregation levels for formulation containing 140mg/mL of the anti-FcRn antibody were similar to those containing 100mg/mL. Methionine-containing formulations showed a high degree of aggregate formation at both concentrations of the anti-FcRn antibody.

High molecular weight (HMW) aggregates were also investigated. The percentage aggregates determined by DSL using the percentage mass size distribution was shown per each of the lyophilised formulations, before lyophilisation and on formulations reconstituted just after lyophilisation (no storage; t0); after reconstitution of the lyophilised pharmaceutical compositions after storage for 3 months at 30°C (t3m, 30°C); after 6 months at 30°C (t6m, 30°C); after 4 weeks at 40°C (t4w, 40°C) and after 3 months at 40°C (t3m, 40°C). Results are shown in

Figures 9A and 9B. Except for methionine-containing formulations, no significant increase in high molecular weight (HMW) aggregates, as measured by DLS, was observed. The increase in HMW aggregates for methionine formulations correlates well with the SE-UPLC data. Aggregation appears to be induced by thermal stress and not by freeze-drying.

Charge variants

Charge variant analysis was carried out as per Example 1. Measurements were performed on pharmaceutical compositions prior lyophilisation, on formulations reconstituted just after lyophilisation (no storage; t0); after reconstitution of the lyophilised pharmaceutical compositions after storage for 3 months at 30°C (t3m, 30°C); after 6 months at 30°C (t6m, 30°C); after 4 weeks at 40°C (t4w, 40°C) and after 3 months at 40°C (t3m, 40°C). For the formulations comprising 100mg/mL of the anti-FcRn antibody, a decrease in acidic species and an increase in basic species were observed for the methionine formulations after storage at 30°C and 40°C (Figures 10A and 10B). A similar behaviour was confirmed for the formulations comprising 140mg/mL of the anti-FcRn antibody and methionine (Figures 11A and 11B). This suggests thermally induced chemical changes in the anti-FcRn antibody due to the presence of methionine, which may also be the reason for the aggregation behavior of the anti-FcRn antibody. For all other formulations no significant changes in charge variants were observed.

Viscosity

Viscosity of reconstituted product was measured with a ViscoPro2000™ viscometer (Cambridge Viscosity™). Measurements were performed on formulation reconstituted just after lyophilisation (no storage). Viscosity was very low (<6cP) for arginine containing formulations, even at 140mg/mL (Figure 12). For all other formulations, the viscosity was higher than >8cP.

Osmolality

Osmolality of reconstituted formulations was measured with a Vapro™ 5520 vapor pressure osmometer (Wescor™). Measurements were performed on formulation reconstituted just after lyophilasition (no storage). All formulations with good stability and reconstitution characteristics, such as the formulations comprising ≥ 7% sucrose showed acceptable osmolality. Remarkably, methionine-containing formulations showed a very low osmolality.

Claims

1. A pharmaceutical composition comprising:
    a. an antibody or an antigen-binding fragment thereof, comprising a light variable region as defined in SEQ ID NO: 7 and a heavy variable region as defined in SEQ ID NO:8;
    b. from 1% to 20% w/v sucrose;
    c. an amino acid or a mixture of amino acids; and
    d. a surfactant, and
    e. a pH of from 4.0 to 7.5.
2. The pharmaceutical composition according to claim 1, wherein the antibody or antigen-binding fragment thereof is at a concentration of from 1 to 200 mg/mL, preferably from 50 to 150 mg/mL, more preferably from 80 to 140mg/mL.
3. The pharmaceutical composition according to any one of the preceding claims, wherein the composition comprises from 5% to 13% w/v sucrose, preferably from 7% to 10% w/v sucrose, more preferably 7% w/v sucrose.
4. The pharmaceutical composition according to any one of the preceding claims, wherein:
    a. the amino acid is histidine or proline; or
    b. the mixture of amino acids comprises histidine and proline.
5. The pharmaceutical composition according to any one of the preceding claims, wherein the composition comprises 0.01 to 1.0%w/v, preferably from 0.01% to 0.5% w/v of the surfactant.
6. The pharmaceutical composition according to claim 5, wherein the surfactant is polysorbate 80.
7. The pharmaceutical composition according to any one of the preceding claims, wherein the antibody is a human or humanised antibody.
8. The pharmaceutical composition according to any one of the preceding claims, wherein the composition comprises from 1mg/mL to 200mg/mL of antibody or antigen-binding fragment thereof, from 10 mM to 50mM histidine, from 10 to 300mM proline, from 1% to 20% w/v sucrose, from 0.01% to 1.0% w/v polysorbate 80, at pH 5.6; preferably the composition comprises from 50mg/mL to 150mg/mL of antibody or antigen-binding fragment thereof, from 20 mM to 40mM histidine, from 50 to 280mM proline, from 5% to 13% w/v sucrose, from 0.01% to 0.5% w/v polysorbate 80, at pH 5.6; more preferably the composition comprises from 80mg/mL to 140mg/mL of antibody or antigen-binding fragment thereof, 30 mM histidine, 180mM proline, 7% w/v sucrose, 0.03% w/v polysorbate 80, at pH 5.6; and even more preferably the composition comprises 100mg/mL of antibody or antigen-binding fragment thereof, 30 mM histidine, 180mM proline, 7% w/v sucrose, 0.03% w/v polysorbate 80, at pH 5.6.

9. The pharmaceutical composition according to any one of the preceding claims, wherein the composition further comprises mannitol and/or arginine and/or glycine.
10. A freeze-dried formulation comprising an antibody or an antigen-binding fragment thereof, sucrose, an amino acid or a mixture of amino acids, and optionally a surfactant, wherein the formulation comprises from 10% to 40% w/w sucrose, preferably from 20% to 40% w/w sucrose and wherein the antibody or antigen-binding fragment thereof comprises a light variable region as defined in SEQ ID NO: 7 and a heavy variable region as defined in SEQ ID NO: 8.
11. A liquid pharmaceutical formulation comprising the freeze-dried formulation according to claim 10 and a solvent.
12. The liquid pharmaceutical formulation according to claim 11, wherein the solvent is water.
13. A container comprising the freeze-dried formulation according to claim 101 or the liquid pharmaceutical formulation according to any one of claims 11 or 12.
14. The pharmaceutical composition according to any one of claims 1 to 9, the freeze-dried formulation according to claim 10 or the liquid pharmaceutical formulation according to any one of claims 11 to 12 for use in therapy.
15. The pharmaceutical composition according to any one of claims 1 to 9, the freeze-dried formulation according to claim 10 or the liquid pharmaceutical formulation according to any one of claims 11 to 12 for use in the treatment of inflammatory or autoimmune diseases selected from myasthenia gravis, Pemphigus vulgaris, Neuromyelitis optica, Guillain-Barré syndrome, lupus, and thrombotic thrombocytopenic purpura, Idiopathic thrombocytopenic purpura (ITP), Chronic inflammatory demyelinating polyneuropathy (CIDP) and combinations thereof.
16. Use of a pharmaceutical composition according to any one of claims 1 to 9, use of the freeze-dried formulation according to claim 10 or use of the liquid pharmaceutical formulation according to any one of claims 11 to 12 in the manufacturing of a medicament for treating an inflammatory or autoimmune disease in a mammalian subject, wherein the inflammatory or autoimmune disease is selected from myasthenia gravis, Pemphigus vulgaris, Neuromyelitis optica, Guillain-Barré syndrome, lupus, and thrombotic thrombocytopenic purpura, Idiopathic thrombocytopenic purpura (ITP), Chronic inflammatory demyelinating polyneuropathy (CIDP) and combinations thereof.
17. A method for reconstituting a pharmaceutical composition according to claim 10 by adding a solvent, wherein the solvent is preferably water.
18. A liquid pharmaceutical formulation obtained by the method according to claim 17.