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(54) Titre : FORMULATIONS LIQUIDES PHARMACEUTIQUES STABLES DE LA PROTEINE DE FUSION TNFR:Fc
(54) Title: STABLE PHARMACEUTICAL LIQUID FORMULATIONS OF THE FUSION PROTEIN TNFR:Fc

(57) **Abrégé/Abstract:**

The present invention relates to stable pharmaceutical liquid formulations of the fusion protein TNFR:Fc comprising different buffer systems and stabilizers. In particular, it could be demonstrated that the physical stability of TNFR:Fc is significantly improved by using a citrate buffer system and lysine as stabilizer.

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(57) Abstract: The present invention relates to stable pharmaceutical liquid formulations of the fusion protein TNFR:Fc comprising different buffer systems and stabilizers. In particular, it could be demonstrated that the physical stability of TNFR:Fc is significantly improved by using a citrate buffer system and lysine as stabilizer.



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Stable pharmaceutical liquid formulations of the fusion protein TNFR:Fc

The present invention relates to stable pharmaceutical liquid formulations of the fusion protein TNFR:Fc comprising different buffer systems and stabilizers. In particular, it could be demonstrated that the physical stability of TNFR:Fc is significantly improved by using a citrate buffer system and lysine and/or proline as stabilizer.

BACKGROUND OF THE INVENTION

Commercial antibodies are commonly formulated in phosphate buffer. Also TNFR:Fc is commonly buffered in sodium phosphate (EP1478394, WO 03/072060 A2). Currently, e.g. the TNFR:Fc protein Etanercept is marketed under the tradename Enbrel[®] having a composition as shown in Table 1.

Table 1 Composition of Etanercept (Enbrel[®])

	[mg/mL]	[mM]
Etanercept	50	0,3
Sucrose	10	29
Sodium chloride	5.8	100
L-arginine hydrochloride	5.3	25
Sodium phosphate monobasic	2.6	19
Sodium phosphate dibasic	0.9	6
WFI	ad 0.5 / 1.0 ml	
pH	6.3 ± 0.2	

Aggregation of antibody products can be controlled by the addition of small amphiphilic molecules. Thereof, L-arginine is the amino acid of choice in suppressing protein interactions in commercial formulations (Baynes et al (2005) 44(12):4919-25; EP1478394). Being a polar additive, it prevents the aggregation of protein folding intermediates.

Like L-arginine, L-lysine is capable of significantly preventing heat- and dilution-induced aggregation of lysozyme (Shiraki et al (2002) J Biochem, 132(4):591-5). L-proline has been established as a stabilizer in liquid immunoglobulin preparations like Sandoglobulin[®] or Privigen[®]. As an hydrophobic amino acid, it is assumed to

interfere with hydrophobic protein-protein interactions and thus protects IgG from denaturation and aggregation. Besides, L-proline exhibits a good safety record when administered to patients with primary immunodeficiencies and was found to represent an amino acid of low toxicity in animal studies (Bolli et al, (2001) Biologicals, 38(1):150-7).

Recent studies contemplate citrate buffer as beneficial in monoclonal antibody formulations as it efficiently minimizes degradations like asparagine deamidations (Zheng and Janis, (2006) Int J Pharm, 308(1-2):46-51). Another advantage of citrate buffer is its capacity to stabilize pH during freezing while the established phosphate buffer system shows the greatest change in pH when lowering temperatures from +25 to -30 degrees C (Kolhe et al, (2010) Biotechnol Prog, 26(3):727-33).

Fusion proteins may generate a variety of degraded and aggregated products which subsequently may lead to reduced activity and even adverse effects like immunogenicity. Thus, there is still a need for a stable liquid formulation of the fusion protein TNFR:Fc.

Such a formulation shall fulfil a variety of tasks. It has to be physiologically acceptable and preferably provides an environment which guarantees stability of the biopharmaceutical drug in a therapeutically effective concentration. Furthermore, the formulation shall enable a satisfactory shelf-life of the drug.

It is thus the object of the present invention to provide pharmaceutical formulations for TNFR:Fc which can be used as an alternative to those formulations known from prior art. Another object of the present invention is to provide pharmaceutical formulations for TNFR:Fc which are advantageous compared to formulations known from prior art. It is yet another object of the present invention to provide pharmaceutical formulations for TNFR:Fc which cause less drug aggregation than formulations known from prior art.

The present invention demonstrates that, by replacing, e.g., the commonly used phosphate buffer to a citrate buffer system and the stabilizer arginine to lysine, the physical stability of TNFR:Fc can be significantly improved. The proposed buffer citric acid and stabilizer lysine protect TNFR:Fc against degradation induced by mechanical and temperature stress (25 and 40°C) and at intended storage at 2-8°C protein degradation was significantly lower in proposed formulations compared to the commonly used phosphate buffered formulations. Therefore, the quality parameters relating to physical stability of the product could be improved. The increased physical stability of the drug product enables a prolonged shelf-life compared to the common product formulations and ensures product safety.

SUMMARY OF THE INVENTION

In a first aspect, the invention relates to a pharmaceutical composition, comprising TNFR:Fc, a citrate buffer and an amino acid selected from the group consisting of lysine and proline and their pharmaceutical acceptable salts.

5 In an embodiment, there is provided a pharmaceutical composition, comprising TNFR:Fc, a citrate buffer at a concentration from 25 mM to 120 mM, and an amino acid at a concentration from 15 mM to 100 mM, wherein the amino acid is lysine or a pharmaceutically acceptable salt thereof and/or proline or a pharmaceutically acceptable salt thereof.

10 In a second aspect, the invention pertains to a kit comprising a composition according to the first aspect and instructions for use of said composition.

In still a third aspect, the invention relates to a method of producing a pharmaceutical composition according to the first aspect, comprising combining TNFR:Fc, a citrate buffer and an amino acid selected from the group consisting of
15 lysine and proline and their pharmaceutical acceptable salts.

In an embodiment, there is provided a method of producing a pharmaceutical composition described herein, comprising combining TNFR:Fc, a citrate buffer at a concentration from 25 mM to 120 mM and an amino acid at a concentration from 15 mM to 100 mM, wherein the amino acid is lysine or a pharmaceutically
20 acceptable salt thereof and/or proline or a pharmaceutically acceptable salt thereof.

In a final aspect, the invention also relates to a composition according to the first aspect for use in a medical treatment, in particular in a treatment of a disease selected from an autoimmune disease, ankylosing spondylitis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis, rheumatoid arthritis, Wegener's disease
25 (granulomatosis), Crohn's disease (or inflammatory bowel disease), chronic obstructive pulmonary disease (COPD), Hepatitis C, endometriosis, asthma, cachexia, atopic dermatitis, Alzheimer and cancer.

In a further aspect, the invention also relates to a use of a composition described herein for treatment of diseases, wherein the diseases are autoimmune disease,
30 ankylosing spondylitis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis, rheumatoid arthritis, Wegener's disease (granulomatosis), Crohn's disease or inflammatory bowel disease, chronic obstructive pulmonary disease (COPD),

Hepatitis C, endometriosis, asthma, cachexia, atopic dermatitis, Alzheimer, cancer, or any combination thereof.

In a further aspect, the invention also relates to a use of a composition described herein for the preparation of a medicament for treatment of diseases, wherein the
5 diseases are autoimmune disease, ankylosing spondylitis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis, rheumatoid arthritis, Wegener's disease (granulomatosis), Crohn's disease or inflammatory bowel disease, chronic obstructive pulmonary disease (COPD), Hepatitis C, endometriosis, asthma, cachexia, atopic dermatitis, Alzheimer, cancer, or any combination thereof.

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DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The formation of degradation products during storage of TNFR:Fc seems to be the most critical attribute of the molecule. TNFR:Fc in citrate formulations displays in general a lower degradation potential, which could be due to the greater net charge
15 of sodium citrate compared to sodium phosphate and therefore possible interaction with the charged TNFR:Fc molecule.

Accordingly, in a first aspect, the invention relates to a pharmaceutical composition, comprising TNFR:Fc, a citrate buffer and an amino acid selected from the group consisting of lysine and proline and their pharmaceutical acceptable salts.

20 Tumor Necrosis Factor alpha (TNF-alpha) is a member of a group of cytokines that stimulate the acute phase reaction, and thus is a cytokine involved in systemic inflammation. TNF-alpha is able to induce inflammation, induce apoptotic cell death, and to inhibit tumorigenesis and viral replication. Dysregulation of TNF-alpha production has been implicated in a variety of human diseases like autoimmune
25 disease, ankylosing spondylitis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis, rheumatoid arthritis, Wegener's disease (granulomatosis), Crohn's disease or inflammatory bowel disease, chronic obstructive pulmonary disease (COPD),

Hepatitis C, endometriosis, asthma, cachexia, atopic dermatitis, Alzheimer as well as cancer.

Its receptor molecules include, TNFR1 (TNF receptor type 1; CD120a; p55/60; for human: RefSeq (mRNA): NM_001065, RefSeq (protein): NP_001056 (SEQ ID NO:1)) and TNFR2 (TNF receptor type 2; CD120b; p75/80; for human: RefSeq (mRNA): NM_001066, RefSeq (protein): NP_001057 (SEQ ID NO:2)). TNF-R1 is expressed in most tissues and can be fully activated by both the membrane-bound and soluble trimeric forms of TNF, whereas TNF-R2 is found only in cells of the immune system and responds to the membrane-bound form of the TNF homotrimer. Upon contact with TNF-alpha, TNF receptors form trimers and thereby initiate intracellular cell signaling.

Accordingly, soluble TNFR molecules or fragments thereof, which are able to bind to TNF-alpha, can be used as a competitive inhibitor for TNF-alpha. The present invention relates to such soluble TNFR molecules fused to an Fc portion of a human immunoglobulin (TNFR:Fc).

In the context of the present invention, the TNFR part of TNFR:Fc refers to any TNFR polypeptide having at least 90%, preferably at least 91 %, such as at least 92 % or at least 93 %, more preferably at least 94 %, such as at least 95 %, or at least 96 %, even more preferably at least 97 %, such as at least 98 %, or at least 99 %, and most preferably 100 % identity to an amino acid sequence comprising at least 150-250, preferably at least 175-245 of TNFR1 or TNFR2, preferably TNFR2, more preferably 200-240, and most preferably 225-235 amino acids of the extracellular part of TNFR2, and still binding to TNF-alpha, as determined by ELISA or any other convenient assay. More preferably, the said TNFR is capable of binding to TNF-alpha and Lymphotoxin alpha (LT-alpha), as determined by ELISA or any other convenient assay. Such assays are well-known to the skilled person.

Generally, a polypeptide has "at least x % identity" over a defined length of amino acids with another polypeptide if the sequence in question is aligned with the best matching sequence of the amino acid sequence and the sequence identity between those to aligned sequences is at least x %. Such an alignment can be performed using for example publicly available computer homology programs such as the "BLAST" program, such as "blastp" provided at the NCBI homepage at <http://www.ncbi.nlm.nih.gov/blast/blast.cgi>, using the default settings provided

therein. Further methods of calculating sequence identity percentages of sets of polypeptides are known in the art.

The Fc-region (fragment crystallisable region) refers to the tail region of an antibody, in the case of IgG composed of the second and third constant domain of the antibody's two heavy chains. In certain embodiments, the Fc polypeptide comprises the constant region of an IgG class heavy chain or a fragment and/or variant thereof and in other embodiments the constant region of other immunoglobulin isotypes can be used to generate such TNFR:Fc fusions. For example, a TNFR:Fc polypeptide comprising the constant region of an IgM class heavy chain or a fragment and/or variant thereof could be used. Preferably, the Fc fragment is derived from IgG, more preferably from IgG1, even more preferably from human IgG1. The constant region of immunoglobulin heavy chains, with a specific example of a human IgG1 class heavy chain constant domain provided by SEQ ID NO: 3, comprises a CH1 domain (amino acids 1 through 98 of SEQ ID NO:3), a hinge region (amino acids 99 through 110 of SEQ ID NO:3), a CH2 domain (amino acids 111 through 223 of SEQ ID NO:3), and a CH3 domain (amino acids 224 through 330 of SEQ ID NO:3). As used herein, an Fc domain can contain one or all of the heavy chain CH1, hinge, CH2, and CH3 domains described above, or fragments or variants thereof. Certain embodiments of the invention include TNFR:Fc comprising all or a portion of the extracellular domain of TNFR1 (SEQ ID NO: 1) or TNFR2 (SEQ ID NO:2) fused to all or a portion of SEQ ID NO:3, optionally with a linker polypeptide between the TNFR portion and the Fc portion of the TNFR:Fc. For example, CH1, CH2 and the entire hinge region may be present in the molecule. In further embodiments, a heavy chain constant region comprising at least a portion of CH1 is the Fc portion of a TNFR:Fc. Certain embodiments can also include, for example, all of the hinge region or the C-terminal half of the hinge region to provide a disulfide bridge between heavy chains. For example, CH1 may be present along with the first seven amino acids of the hinge (amino acids 99 through 105 of SEQ ID NO: 3). In certain embodiments of this invention, the TNFR polypeptide is covalently linked, optionally through a polypeptide linker, to the N-terminus of at least one portion of a CH1 region of a heavy chain constant domain to form a TNFR:Fc.

If a dimeric TNFR:Fc is desired, it is important to include the portion of the hinge region implicated in disulfide bond formation between the heavy chains (for example, a portion of amino acids 99 through 110 of SEQ ID NO: 3 that includes amino acid

109 of SEQ ID NO: 3). In further embodiments of the invention, the TNFR:Fc can comprise portions of the CH3 domain that do not include the C-terminal lysine residue (amino acid 330 of SEQ ID NO: 3), as this residue has been observed to be removed in post-translational processing of IgG heavy chain polypeptides. Fc fusions and Fc fragments are well-known in the art.

Preferably, the TNFR:Fc is essentially identical / similar to Etanercept, more preferably, the TNFR:Fc is Etanercept.

Etanercept is a dimer of two molecules of the extracellular portion of the p75 TNF-alpha receptor, each molecule consisting of a 235 amino acid TNFR-derived polypeptide that is fused to a 232 amino acid Fc portion of human IgG1. The amino acid sequence of the monomeric component of etanercept is shown as SEQ ID NO:4. In the dimeric form of this molecule, two of these fusion polypeptides (or "monomers") are held together by three disulfide bonds that form between the immunoglobulin portions of the two monomers. The etanercept dimer therefore consists of 934 amino acids, and has an apparent molecular weight of approximately 150 kilodaltons. In North America, etanercept is co-marketed by Amgen and Pfizer under the trade name Enbrel® in two separate formulations, one in powder form, the other as a pre-mixed liquid. Wyeth is the sole marketer of Enbrel® outside of North America excluding Japan where Takeda Pharmaceuticals markets the drug.

The term "essentially identical / similar to Etanercept" as used herein means that the amino acid sequence of the TNFR:Fc has at least 95% identity to the amino acid sequence shown in SEQ ID NO: 4, more preferably at least 96% identity, such as 97% identity, and most preferably 98% identity, such as 99% identity to the amino acid sequence shown in SEQ ID NO: 4. Alternatively or additionally, the TNFR:Fc may (only) differ from Etanercept by postranslational modifications, e.g. by glycosylation. Suitable procedures for changing a glycosylation pattern, such as introducing or deleting a glycosylation site, and tests for determining a glycosylation pattern are well known to the skilled person.

The TNFR:Fc may be recombinantly produced, preferably by using a mammalian cell based expression system. Preferably, said mammalian cell-based expression system is at least one selected from the group consisting of Baby hamster Kidney cell lines (e.g., BHK21); Chinese hamster ovary cell lines (e.g., CHO-K1, CHO-DG44, CHO-DXB, or CHO-dhfr-); Murine myeloma cell lines (e.g., SP2/0); Mouse myeloma cell lines (e.g., NS0); Human embryonic kidney cell lines (e.g., HEK-293); Human-retina-

derived cell lines (e.g., PER-C6), and/or Amniocyte cell lines (e.g., CAP). Preferably, hamster cell based expression systems are being used. BHK21 ("Baby Hamster Kidney") cells belong to a quasi diploid established line of Syrian hamster cells, descended from a clone from an unusually rapidly growing primary culture of newborn hamster kidney tissue. Non limiting examples for BHK-21 cell lines which are commercially available and can be used in the context of the present invention are BHK-21 (C-13); BHK21-pcDNA3.1-HC; BHK570; Flp-In-BHK Cell Line; and/or BHK 21 (Clone 13) hamster cell line.

Chinese hamster ovary (CHO) cells are a cell line derived from the ovary of the Chinese hamster. They are often used in biological and medical research and are commercially utilized in the production of therapeutic proteins. They were introduced in the 1960s and were originally grown as a monolayer culture. Today, CHO cells are the most commonly used mammalian hosts for industrial production of recombinant protein therapeutics and are usually grown in suspension culture.

Non limiting examples for CHO cell lines which are commercially available and can be used in the context of the present invention are FreeStyle CHO-S cells; ER-CHO Cell Line; CHO 1-15 500 CHINESE HAM; CHO-DXB, CHO-dhfr-, CHO DP-12 clone#1934; CHO-CD36; CHO-ICAM-1; CHO-K1; Ovary; HuZP3-CHOLec3.2.8.1; xrs5; CHO-K1/BB2 Cells; CHO-K1/BB3 Cells; CHO-K1/EDG8/Galpha15 Cells; CHO-K1/M5 Cells; CHO-K1/NK1 Cells; CHO-K1/NK3 Cells; CHO-K1/NMUR1 Cells; CHO-K1/NTSR1 Cells; CHO-K1/OX1 Cells; CHO-K1/PAC1/Gα15 Cells; CHO-K1/PTAFR Cells; CHO-K1/TRH1 Cells; CHO-K1/V1B Cells; 5HT1A Galpha-15-NFAT-BLA CHO-K1 Cell Line; AVPR2 CRE-BLA CHO-K1 Cell Line; CHO-S Cells SFM Adapted; DG44 Cells; Flp-In-CHO Cell Line; GeneSwitch-CHO Cell Line; NFAT-bla CHO-K1 Cell Line; T-REx-CHO Cell Line; GenoStat CHO K-1 Stable Cell Line; GenoStat CHO K-1 Stable Cell Line Kit; CHO-K1 Cell Line hamster, CHO-PEPT1 Cell line, CHO SSF3 and/or CHO-HPT1 Cell Line. In a particularly preferred embodiment, the hamster cell-based expression system is a CHO-dhfr-cell line.

The pharmaceutical composition may comprise TNFR:Fc at a concentration from 0.1 mM to 0.7 mM, such as 0.2 mM or 0.6 mM, preferably at a concentration from 0.15 mM to 0.5 mM, such as 0.4 mM or 0.45 mM, more preferably at a concentration from 0.25 mM to 0.35 mM, such as about 0.3 mM.

The citrate buffer may be any suitable citrate buffer. For example, the citrate buffer may comprise or consist of sodium citrate, potassium citrate, citric acid, or mixtures

thereof. The citrate buffer seems to have the greatest influence on the stability of the formulation. The increased stability of a 50 mM citrate buffer formulation compared to 25 mM citrate buffer formulation is shown in the examples. An increase up to at least 120 mM could lead to even increased effects. A minimum of 25 mM citrate buffer seems to be mandatory for the stabilization. Accordingly, the pharmaceutical composition may comprise the citrate buffer at a concentration from 25 mM to 120 mM, such as from 30 mM to 115 mM, preferably at a concentration from 40 mM to 110 mM, such as from 45 mM to 105 mM, more preferably at a concentration from 50 mM to 100 mM, such as at a concentration of 55 mM, 60 mM, 65 mM, 70 mM, 75 mM, 80 mM, 85 mM, 90 mM, or 95 mM. In another preferred embodiment, the pharmaceutical composition comprises the citrate buffer at a concentration as indicated in the compositions described in the Examples section.

The pH is preferably a value of 5 to 7.5, such as from 5.5 to 7, even more preferably from 6 to 6.6, such as 6.1 to 6.5, most preferably from 6.2 to 6.4, such as about 6.3. It is noted that although the pH is preferably a value of 5 to 7.5, alternatively lower pH values of up to 2.8, e.g. in the range of 2.8 to 4.0 may alternatively also be employed. The pharmaceutical composition comprises an amino acid selected from the group consisting of lysine and proline and their pharmaceutical acceptable salts, such as hydrochlorides. The amino acid may be in D-, L- or DL-configuration, preferably in L-configuration.

At the addition of about 25 mM the additional effect of the combination with the basic amino acid lysine is striking. The addition of up to 100 mM lysine is believed to have an additional effect. Thus, the pharmaceutical composition may comprise the amino acid at a concentration from 15 mM to 100 mM, preferably at a concentration from 20 mM to 90 mM, more preferably at a concentration from 25 mM to 75 mM. In another preferred embodiment, the pharmaceutical composition comprises the amino acid at a concentration as indicated in the compositions described in the Examples section. In a particularly preferred embodiment, the amino acid is lysine, or its pharmaceutical acceptable salts.

In addition, the pharmaceutical composition may further comprise at least one tonicity modifier. As used herein, the term "tonicity modifier" is intended to describe a molecule other than citrate, lysine or proline that contributes to the osmolality of a solution. Preferably, the osmolality of a pharmaceutical composition is regulated in order to stabilize the active ingredient and in order to minimize the discomfort to the

patient upon administration. Generally, it is preferred that a pharmaceutical composition be isotonic with serum by having the same or similar osmolality, i.e. an osmolality from about 180 to 480 mosmol/kg. Preferably, the at least one tonicity modifier is selected from the group consisting of sodium chloride, cysteine, histidine, glycine, potassium chloride, sucrose, glucose and mannitol, more preferably the tonicity modifier is sodium chloride and/or sucrose. The pharmaceutical composition may comprise the at least one tonicity modifier at a total concentration from 5 mM to 200 mM, such as from 10 mM to 190 mM, from 15 mM to 180 mM, from 20 mM to 170 mM, from 25 mM to 160 mM, from 30 mM to 150 mM, from 35 mM to 140 mM, from 40 mM to 130 mM, or from 45 mM to 120 mM, e.g. 110 mM, but preferably at a concentration from 50 mM to 100 mM. In another preferred embodiment, the pharmaceutical composition comprises the tonicity modifier(s) at a concentration as indicated in the compositions described in the Examples section.

Further, the pharmaceutical composition may comprise at least one excipient. The term "excipient" as used herein refers to a pharmacologically inactive substance used as a carrier for the active agent in a pharmaceutical composition. In some cases, an "active" substance may not be easily administered and absorbed by the human body. In such cases the substance in question may be mixed with an excipient or dissolved in an excipient solution. Excipients are also sometimes used to bulk up formulations that contain very potent active ingredients, in order to facilitate a convenient and accurate dosing. In addition to their use in the single-dosage quantity, excipients can be used in the manufacturing process to optimize the handling of the concerned active substance. Depending on the route of administration and the form of the pharmaceutical composition, different excipients may be used. Thus, excipients may comprise inter alia antiadherents, binders, colours and preservatives such as antioxidants.

For example, at least one excipient may be selected from the group consisting of lactose, glycerol, xylitol, sorbitol, mannitol, maltose, inositol, trehalose, glucose, bovine serum albumin (BSA), dextran, polyvinyl acetate (PVA), hydroxypropyl methylcellulose (HPMC), polyethyleneimine (PEI), gelatin, polyvinylpyrrolidone (PVP), hydroxyethylcellulose (HEC), polyethylene glycol (PEG), ethylene glycol, glycerol, dimethylsulfoxide (DMSO), dimethylformamide (DMF), L-serine, sodium glutamate, alanine, glycine, sarcosine, gamma-aminobutyric acid (GABA), polyoxyethylene sorbitan monolaurate (Tween™-20), polyoxyethylene sorbitan

monooleate (Tween™-80), sodium dodecyl sulphate (SDS), polysorbate, polyoxyethylene copolymer, potassium phosphate, sodium acetate, ammonium sulfate, magnesium sulfate, sodium sulfate, trimethylamine N-oxide, betaine, zinc ions, copper ions, calcium ions, manganese ions, magnesium ions, CHAPS, sucrose monolaurate and 2-O-beta-mannoglycerate. In one preferred embodiment, the excipient may be chosen from those described in the Examples section.

The pharmaceutical composition may comprise the at least one excipient at a total concentration of at least 0.1 mM, e.g. from 0.1 mM to 0.7 mM, such as at a concentration of 0.6 mM, preferably at a concentration from 0.15 mM to 0.5 mM, such as from 0.2 mM to 0.4 mM, more preferably at a concentration from 0.24 mM to 0.34 mM. In another preferred embodiment, the pharmaceutical composition comprises the excipient at a concentration as indicated in the compositions described in the Examples section.

Preferably, the composition is liquid. However, in another embodiment, the pharmaceutical composition may be lyophilized, and can be reconstituted, for example by the addition of water, forming a liquid composition. Hence, the pharmaceutical composition may further comprise a pharmaceutically acceptable solvent. In a preferred embodiment, the pharmaceutically acceptable solvent is water. The concentrations of components presented herein refer to a liquid formulation as well as to a constituted lyophilate or a formulation to be lyophilised.

Excipients might display a protective effect during freezing of lyophilized formulations, so called cryoprotective features. Furthermore, metal chelating agents and tensides can be added. Some agents may have a double role, e.g., some sugars or sugar alcohols can serve for example as excipient, cryoprotective and/or tonifying agent.

The present formulation in aqueous state is ready to use, while the lyophilized state of the the present formulation can be transferred into liquid formulations by e.g. addition of water for injection.

Particularly preferred compositions comprise or consist of 0.1 mM to 0.7 mM TNFR:Fc, e.g. Etanercept, 25 mM to 120 mM citrate buffer, e.g. sodium citrate, 15 mM to 100 mM lysine, e.g. lysine hydrochloride, 10 mM to 100 mM sucrose and 5 mM to 200 mM sodium chloride at a pH value of about 6.3.

Alternatively, the pharmaceutical composition may comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 50 mM citrate buffer, e.g. sodium citrate, 25 mM lysine,

e.g. lysine hydrochloride, 29 mM sucrose and 75 mM sodium chloride at a pH value of about 6.3.

In still another preferred embodiment, the pharmaceutical composition may comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 25 mM citrate buffer, e.g. sodium citrate, 25 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 88 mM sodium chloride at a pH value of about 6.3.

However, the pharmaceutical composition may also comprise or consist of 0.1 mM to 0.7 mM TNFR:Fc, e.g. Etanercept, 25 mM to 120 mM citrate buffer, e.g. sodium citrate, 15 mM to 100 mM proline, 10 mM to 100 mM sucrose and 5 mM to 200 mM sodium chloride at a pH value of about 6.3.

Finally, the pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 25 mM citrate buffer, e.g. sodium citrate, 25 mM proline, 29 mM sucrose and 75 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 50 mM citrate buffer, e.g. sodium citrate, 50 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 75 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 120 mM citrate buffer, e.g. sodium citrate, 25 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 75 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 120 mM citrate buffer, e.g. sodium citrate, 50 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 75 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 50 mM citrate buffer, e.g. sodium citrate, 50 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 51 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 120 mM citrate buffer, e.g. sodium citrate, 25 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 22 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 120 mM citrate buffer, e.g. sodium citrate, 50 mM lysine, e.g. lysine hydrochloride and 29 mM sucrose at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 50 mM citrate buffer, e.g. sodium citrate, 50 mM lysine, e.g. lysine hydrochloride and 75 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 120 mM citrate buffer, e.g. sodium citrate, 25 mM lysine, e.g. lysine hydrochloride and 36 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 120 mM citrate buffer, e.g. sodium citrate, 50 mM lysine, e.g. lysine hydrochloride and 17 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 50 mM citrate buffer, e.g. sodium citrate, 50 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 75 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 75 mM citrate buffer, e.g. sodium citrate, 25 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 75 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 75 mM citrate buffer, e.g. sodium citrate, 50 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 75 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 75 mM citrate buffer, e.g. sodium citrate, 25 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 56 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 75 mM citrate buffer, e.g. sodium citrate, 50 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 31 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 75 mM citrate buffer, e.g. sodium citrate, 25 mM lysine, e.g. lysine

hydrochloride, 19 mM sucrose and 75 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 75 mM citrate buffer, e.g. sodium citrate, 50 mM lysine, e.g. lysine hydrochloride and 59 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 25 mM citrate buffer, e.g. sodium citrate, 25 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 75 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 25 mM citrate buffer, e.g. sodium citrate, 50 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 75 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 50 mM citrate buffer, e.g. sodium citrate, 25 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 75 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 50 mM citrate buffer, e.g. sodium citrate, 50 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 75 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 50 mM citrate buffer, e.g. sodium citrate, 50 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 48 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 25 mM citrate buffer, e.g. sodium citrate, 25 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 88 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 25 mM citrate buffer, e.g. sodium citrate, 25 mM proline, 29 mM sucrose and 88 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 25 mM citrate buffer, e.g. sodium citrate, 25 mM proline, 29 mM sucrose and 100 mM sodium chloride at a pH value of about 6.3.

5 The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 25 mM phosphate buffer, e.g. sodium citrate, 25 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 88 mM sodium chloride at a pH value of about 6.3.

10 The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 25 mM phosphate buffer, e.g. sodium citrate, 25 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 75 mM sodium chloride at a pH value of about 6.3.

15 The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 50 mM citrate buffer, e.g. sodium citrate, 50 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 50 mM sodium chloride at a pH value of about 6.3.

The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 75 mM citrate buffer, e.g. sodium citrate, 25 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 50 mM sodium chloride at a pH value of about 6.3.

20 The pharmaceutical composition may also comprise or consist of 0.3 mM TNFR:Fc, e.g. Etanercept, 65 mM citrate buffer, e.g. sodium citrate, 25 mM lysine, e.g. lysine hydrochloride, 29 mM sucrose and 55 mM sodium chloride at a pH value of about 6.3.

25 Preferably, the pharmaceutical composition is a stable liquid composition. The term "stable" as used herein means that the TNFR:Fc exhibits one or more of the following features:

30 (i) exhibiting less than 99% aggregation products (SUM APs) as compared to the same TNFR:Fc formulated in the commonly used phosphate buffered formulation containing 0,3 mM Etanercept in a matrix consisting of 25 mM phosphate buffer, 25 mM arginine and sodium chloride in a molarity greater than 75 mM or in a the amount needed to adjust isotonicity, more preferably less than 98,5% SUM APs, even more preferably less than 98% SUM APs, most preferably less than 97,5% SUM APs, as determined after three months of storage at 40°C by SEC;

(ii) and/or exhibiting less than 99% degradation products (SUM DPs) as compared to the same TNFR:Fc formulated in the commonly used phosphate buffered formulation containing 0,3 mM etanercept in a matrix consisting of 25 mM phosphate buffer, 25 mM Arginine and sodium chloride in a molarity greater than 75 mM or in a the amount needed to adjust isotonicity, more preferably less than 98% SUM DPs, such as less than 97% SUM DPs, even more preferably less than 96% SUM DPs, such as less than 95% SUM DPs, most preferably less than 94% SUM DPs, such as less than 93% SUM DPs, as determined after three months of storage at 40°C by SEC.

Thus, the pharmaceutical formulation according to the invention may be suitably formulated for long term storage. As used herein, the term "long term storage" shall refer to storage of a composition comprising the pharmaceutical formulation for more than 1 month, preferably for more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or even 12 months.

As exemplified below, stability of formulations containing either a citrate or phosphate buffer system in combination with either lysine or proline as stabilizer were assessed during a three month-stability study at intended (2-8 °C) as well as accelerated storage condition (25 and 40°C). Formulations containing the citrate buffer system were thereby determined to be superior compared to formulations containing the phosphate buffer system regarding the formation of post peaks (as determined e.g. by RPC), the formation of degradation products (as determined e.g. by SEC) and the formation of acid peaks (as determined e.g. by CEX).

The described effects were partially even more pronounced, including the superior stabilization of TNFR:Fc formulated in the citrate/lysine formulation matrix, if formulations were stored in vials, displaying a greater liquid/air interaction surface. Determination of the stability of a liquid composition is further exemplified in the examples section, and in particular in Example 3.

The pharmaceutical composition according to the first aspect can be used in a medical treatment. Diseases which may be treated by using the pharmaceutical composition of the invention include, but are not limited to autoimmune diseases, ankylosing spondylitis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis, rheumatoid arthritis, Wegener's disease (granulomatosis), Crohn's disease or inflammatory bowel disease, chronic obstructive pulmonary disease (COPD), Hepatitis C, endometriosis, asthma, cachexia, atopic dermatitis, Alzheimers disease

and cancer. Accordingly, also contemplated is a method of treatment, comprising administering the composition according to the first aspect to a subject in need thereof, e.g. a subject suffering from one of the above mentioned diseases.

Dosage of the TNFR:Fc will depend on the disease, severity of condition, patient's clinical history, and response to the (prior) therapy, and will be adjusted and monitored by a physician. The pharmaceutical composition may be administered parenterally, such as subcutaneously, intramuscularly, intravenously, intraperitoneally, intracerebrospinally, intra-articularly, intrasynovially and/or intrathecally by either bolus injection or continuous infusion.

The dosage of TNFR:Fc per adult may range from about 1-500 mg/m², or from about 1-200 mg/m², or from about 1-40 mg/m² or about 5-25 mg/m². Alternatively, a flat dose may be administered, wherein the amount may range from 2-500 mg/dose, 2-100 mg/dose or from about 10-80 mg/dose. The dose may be administered more than one time per week, such as two or more times per week at a dose range of 25-100 mg/dose. In another embodiment, an acceptable dose for administration by injection contains 80-100 mg/dose, e.g. 80 mg per dose. The doses can be administered weekly, biweekly or separated by several weeks, e.g. by three weeks.

It is further contemplated that an improvement of the patient's condition will be obtained by a dose of up to 100 mg of the pharmaceutical composition one to three times per week over a period of at least three weeks, though treatment for longer periods may be necessary to induce the desired degree of improvement. However, for incurable chronic conditions the regimen may be continued indefinitely. A suitable regimen for paediatric patients (ages 4-17) may involve a dose of 0.4 mg/kg to 5 mg/kg of TNFR:Fc, administered one or more times per week.

More specifically, in the case of rheumatoid arthritis, 25 mg TNFR:Fc may be administered twice weekly. Alternatively, 50 mg administered once weekly has been shown to be safe and effective.

In the case of psoriatic arthritis and ankylosing spondylitis, the recommended dose is 25 mg TNFR:Fc administered twice weekly or 50 mg administered once weekly.

Turning to plaque psoriasis, the recommended dose of TNFR:Fc is 25 mg administered twice weekly or 50 mg administered once weekly. Alternatively, 50 mg given twice weekly may be used for up to 12 weeks followed, if necessary, by a dose of 25 mg twice weekly or 50 mg once weekly. Treatment with TNFR:Fc should continue until remission is achieved, for up to 24 weeks. Continuous therapy beyond

24 weeks may be appropriate for some adult patients. Treatment should be discontinued in patients who show no response after 12 weeks. If re-treatment with TNFR:Fc is indicated, the same guidance on treatment duration should be followed. The dose should be 25 mg twice weekly or 50 mg once weekly.

5 Regarding juvenile idiopathic arthritis (age 4 years and above), 0.4 mg/kg (up to a maximum of 25 mg per dose) after reconstitution of 25 mg TNFR:Fc in 1 ml of solvent, may be given twice weekly as a subcutaneous injection with an interval of 3-4 days between doses.

Concerning paediatric plaque psoriasis (age 8 years and above), 0.8 mg/kg (up to a maximum of 50 mg per dose) once weekly for up to 24 weeks may be administered. Treatment should be discontinued in patients who show no response after 12 weeks. If re-treatment with TNFR:Fc is indicated, the above guidance on treatment duration should be followed. The dose should be 0.8 mg/kg (up to a maximum of 50 mg per dose) once weekly.

15 In case of renal and hepatic impairment no dose adjustment is required.

In a second aspect, the invention relates to a kit comprising a composition according to the first aspect and instructions for use of the present composition.

In a preferred embodiment, the composition is contained in a pre-filled syringe. In another preferred embodiment, the composition is contained in a pre-filled vial. The kit may comprise one or more unit dosage forms containing the pharmaceutical composition of the invention. Examples for suitable syringes are BD Hypak SCF™ 1ml long, glass pre-fillable syringes assembled with PTFE coated stoppers (rubber quality 4023/50 from West). The glass vials may be for example 6R glass vials (hydrolytic class I) assembled with PTFE coated stoppers (rubber quality 4023/50 from West). However, any other suitable syringe or vial may be used. The kit may also comprise the pharmaceutical composition according to the invention in another secondary container, such as in an autoinjector.

The prefilled syringe may contain the formulation in aqueous form. Described syringe may be further supplied with an autoinjector, which often is a disposable article for single use only, and may e.g. have a volume between 0.1 and 1 ml. However, the syringe or autoinjector may also be for multi-usage or multi-dosing. The described vial may contain the formulation in lyophilised or aqueous state, and may serve as a single or multiple use device. The vial may e.g. have a volume between 1 and 10 ml.

In a third aspect, the invention pertains to a method of producing a pharmaceutical composition according to the first aspect, comprising combining TNFR:Fc, a citrate buffer and an amino acid selected from the group consisting of lysine and proline and their pharmaceutical acceptable salts.

5 In one particular embodiment, the method may comprise a further step of adding a pharmaceutically acceptable solvent as defined above. The method may further comprise the step of adding at least one tonicity modifier, such as sodium chloride and/or sucrose, and optionally an excipient as defined above. In a final preferred embodiment, the method may further comprise a lyophilization step, which step may
10 be before or after adding the at least one tonicity modifier, and/or an excipient as defined above.

The invention will be more fully understood by reference to the following examples. However, the examples should not be construed as limiting the scope of the invention.

15 DESCRIPTION OF THE FIGURES

Figure 1. Results for the SEC analysis of TNFR:Fc during focused screening; Abbreviations: Pho = phosphate buffer system; Cit = citrate buffer system; Pro = L-proline; Lys = L-lysine. **A:** Coefficient Plot for degradation products after storage at
20 25°C. **B:** Coefficient Plot for degradation products after storage at 40°C. **C:** Coefficient Plot for degradation products after 48 hours stirring at 600 rpm. **D:** Coefficient Plot for degradation products after three freezing and thawing cycles.

EXAMPLES

25 Description of Materials

The TNFR:Fc which was used for the described examples, is derived from recombinant CHO cells, which have been cultured in a fed-batch process in chemically defined medium. The TNFR:Fc is purified from the cell free harvest by standard methods including affinity chromatography on protein A resins and by
30 further chromatographic and filtration steps.

Description of methods

General

All chromatographic methods were performed on Agilent 1100 and 1200 HPLC systems equipped with UV and fluorescence detection.

5

Size exclusion chromatography (SEC)

Size exclusion chromatography was used to separate lower and higher molecular mass variants of TNFR:Fc as well as any impurities and formulation ingredients. The results were described as the summation of aggregation peaks (APs) and summation of degradation peaks (DPs). In SEC, the identity of test samples was determined by comparing the chromatographic retention time of the major peaks with the retention time of the major peak of a reference standard.

10

SEC was performed using two sequential Tosoh Bioscience TSK-Gel G3000SWXL columns (5 µm, 250 Å, 7.8 mm I.D. x 300 mm length) (Tosoh Bioscience, Stuttgart, Germany) and a mobile phase containing 150 mM potassium phosphate, pH 6.5. The flow rate was set to 0.4 ml/min and the column temperature to 30°C. Samples were diluted with mobile phase to a concentration of 0.75 mg/ml and injection volume was 10 µl.

15

Reverse Phase Chromatography (RP-HPLC or RPC)

The content of samples was determined via RP-HPLC using a C8 column (5 µm, 300 Å, 2.1 mm I.D. x 75 mm length) at 70°C column temperature. Separation of product variants was achieved by applying a linear gradient from 20 to 30% mobile phase B (mobile phase A: 10 % acetonitrile, 0.3 % PEG 300, 0.1 % TFA; mobile phase B: 90 % acetonitrile, 0.3 % PEG 300, 0.1 % TFA) at a flow rate of 1.0 ml/min. Samples were desialylated and diluted to a concentration of 2.5 mg/ml before injection. Chromatograms of UV detection were used for evaluation of purity. Results were displayed as content as well as post peaks (Sum PPs).

25

Cation exchange chromatography (CEX)

Cation-exchange separation was performed using a silica based cation exchange resin with bonded coating of polyaspartic acid (100 mm length x 4.6 mm i.d.; 3 µm particles). Before injection, samples were desialylated and concentration was adjusted to 2.5 mg/ml with desialylation buffer. Samples were eluted by a linear gradient from 30 to 50% mobile phase B over 20 min (A: 50 mM sodium acetate, pH 5.2; B: 50 mM sodium acetate, 250 mM NaCl, pH 5.2). The flow rate and temperature were set to 1.0 ml/min and 30°C, respectively. Chromatograms from UV detection were used for data evaluation. The results were displayed as summation of acidic peaks (Sum APs) and summation of basic peaks (Sum BPs)

35

Particle counting (PC) method

Particle counting was performed by light obscuration, utilizing an Accusizer Nicomb SIS 780 instrument. This technique detects the light scattered by a particle/aggregate within a liquid environment. The signal will be calculated into the hydrodynamic radius for the detected particle/aggregate. A mean out of three measurements was calculated utilizing a total sample volume of 2.8 ml. The following instrument parameters were used: Range: 0.5 – 500 μm (512 channel; logarithmic scale); flow rate: 5 ml/min.

The influence of the excipients sucrose, arginine and NaCl on the aggregation of TNFR:Fc was evaluated prior to performing the tests described in example 1-3. The resulting formulations were subjected to a stress stability study including stirring and shaking. Aggregates with a radius up to 200nm were detected via SEC method, while particle counting determines particles in the range of 500 nm to 400 μm .

Arginine:

Analysis of stressed formulations via SEC, revealed the reduction of aggregates due to the addition of arginine. At the same time the formation of bigger sized particles with increasing molarity of arginine was detected via the PC method. It can therefore be concluded that increasing amounts of arginine in TNFR:Fc formulations facilitate the decrease of smaller sized particles (r = up to 200 nm), which is most probably due to the clustering of aggregates to bigger sized particles which are only detectable via the particle counting method.

NaCl:

The addition of higher NaCl molarities led to the same PC results as for the addition of arginine. The amount of DPs, detected via SEC method, increased with increasing molarities of NaCl

Sucrose:

The addition of increasing molarities of sucrose, seemed to led to the reduction of the dedcted radius of paricles via PC method. No significant results were detected via SEC.

Example 1

Formulation Screening

The evaluation was performed by a combination of a DoE (design of experiments) and single parameter studies. All formulations contained 50 mg/ml TNFR:Fc, 100 mM NaCl and were adjusted to a pH of around pH 6.3. The formulations were divided into

groups called surfactants, buffer, stabilizer I (containing sugars and sugar alcohols) as well as stabilizer II (amino acids), whereas the stabilizer groups were designed and evaluated by a statistical design (Table 3). The results from these groups were evaluated for their statistical significance. Results were in addition compared to the prior art formulation corresponding to Enbrel[®] (Table 1) of the TNFR:Fc.

Table 3. Evaluated factors during formulation screening of TNFR:Fc

<i>Evaluated factors</i>	<i>Applied range</i>
<i>Sugars - Stabilizer I</i>	
Sucrose	0-100 mM
Trehalose	0-100 mM
Sorbit	0-100 mM
<i>Amino acids – Stabilizer II</i>	
Glycine	0-100 mM
Lysine	0-100 mM
Proline	0-100 mM
Arginine	0 / 100 mM
<i>Buffer</i>	
Phosphate	0 / 25 mM
Succinate	0 / 25 mM
Histidine	0 / 25 mM
Citrate	0 / 25 mM
<i>Tonicity agent</i>	
NaCl	100 / 150 mM

The resulting formulations (Table 4) were mechanically stressed and subjected to a short term stress stability study (Table 5). As reference served the Etanercept formulation (i.e. as described in table 1).

Table 4. Formulation evaluated during formulation screening phase. The set up combines a single parameter evaluation of buffers (formulation 1 - 4) as well as a DoE approach for stabilizer group I including sugars or sugar alcohols (formulation 5 - 9) and stabilizer group II including amino acids (formulation 10 - 14). In addition one formulation similar to prior art formulation corresponding to Enbrel® but omitting arginine (formulation 18) as well as one formulation omitting arginine and adding a higher concentration of NaCl (formulation 19) were tested. The prior art formulation corresponding to Enbrel® served ascontrol (formulation 1).

	Buffer				DoE					DoE				Single parameter		
					Stabilizer I [mg/ml]					Stabilizer II [mg/ml]				omitting	omitting Arginine, including NaCl	
														Arginine		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		[mg/ml]														[mg/ml]
Etanercept (M=150000g/mol)	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Sucrose	10	10	10	10	-	17,1	34,2	34,2	-	10	10	10	10	10	10	10
Trehalose	-	-	-	-	-	18,9	37,8	-	37,8	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	18,2	9,1	18,2	-	-	-	-	-	-	-	-	-
NaCl	5,8	5,8	5,8	5,8	5,8	5,8	5,8	5,8	5,8	5,8	5,8	5,8	5,8	5,8	5,8	8,8
Arginine	5,3	5,3	5,3	5,3	-	-	-	-	-	11,5	11,5	5,8	-	-	-	-
Proline	-	-	-	-	-	-	-	-	-	-	14,6	7,3	-	14,6	-	-
Lysine	-	-	-	-	-	-	-	-	-	-	7,5	3,8	7,5	-	-	-
Glycine	-	-	-	-	-	-	-	-	-	-	2,6	2,6	2,6	2,6	2,6	2,6
NaH2PO4	2,6	-	-	-	2,6	2,6	2,6	2,6	2,6	0,9	0,9	0,9	0,9	0,9	0,9	0,9
Na2HPO4	0,9	-	-	-	0,9	0,9	0,9	0,9	0,9	-	-	-	-	-	-	-
DiNatriumSuccinate	-	6,8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Histidine	-	-	3,9	-	-	-	-	-	-	-	-	-	-	-	-	-
Citric acid	-	-	-	5,3	-	-	-	-	-	-	-	-	-	-	-	-
pH	6,3	6,3	6,3	6,3	6,3	6,3	6,3	6,3	6,3	6,3	6,3	6,3	6,3	6,3	6,3	6,3

Table 5. Analytical methods and stress conditions applied during formulation screening phase. Rotations per minute (rpm); Agitation per minute (apm)

<i>Methods</i>	<i>Stress conditions</i>	<i>Pull points</i>
<i>Shaking (180 apm*)</i>		
SEC	Formulation 1 - 20	6 hours
SEC	Formulation 1 - 20	16 hours
<i>Stirring (600 rpm**)</i>		
SEC	Formulation 1 - 20	6 hours
SEC	Formulation 1 - 20	16 hours
<i>Freeze / Thaw cycles</i>		
SEC	Formulation 1, 2, 3 and 20	1 cycle
SEC	Formulation 1, 2, 3 and 20	3 cycles
<i>Temperatures</i>		
SEC, CEX, RPC	Formulation 3 – 20	0 weeks
SEC, CEX, RPC	Formulation 3 – 20	2 weeks
SEC, CEX, RPC	Formulation 3 – 20	4 weeks

5 A positive influence on the quality attributes of TNFR:Fc for the out parameters RPC, CEX and SEC was defined as:

- Content (RPC) – no decrease
- Sum APs (CEX) – low level
- Sum BPs (CEX) – comparable to prior art based formulation
- 10 • Sum APs (SEC) – low level
- Sum DPs (SEC) – low level

Results were in addition compared to the TNFR:Fc in the prior art formulation corresponding to Enbrel® and marked positive if they were at least comparable to the results of the prior art formulation corresponding to Enbrel® or if they were exceeding them according to the above mentioned parameters. A positive influence on the TNFR:Fc stability in formulation showed the evaluated factors proline, lysine, sucrose and citrate (Table 6).

Table 6. Results of formulation screening. The influence of the various factors are described with a minus (-) or plus (+) symbol; - reflects a negative influence compared to the prior art formulation corresponding to Enbrel[®] and/or the expected outcome, whereas + reflects a positive influence compared to the prior art formulation corresponding to Enbrel[®] and/or the expected outcome; bracketing of the result indicates less pronounced effects; non-significant results are not filled; Factors marked bold show a positive impact on product quality; Factors marked italic were further investigated (see example 3); The factors marked with an asterisk (*) show adverse effects and were excluded from further investigations.

Evaluated Factors	RPC	CEX		SEC	
	Content	Sum APs	Sum BPs	Sum APs	Sum DP's
<u>Amino acids</u>					
Glycin (*)		(-)			
Lysine		+		+	-
Proline		+		+	-
<u>Sugars</u>					
Sucrose	(-)	(+)			
<i>Trehalose</i>		+			
Sorbit (*)		(-)			
<u>Buffers</u>					
Phosphate				(-)	(-)
<i>Succinate</i>		+	(+)	+	-
Histidine (*)		+	-	(-)	(-)
Citrate	(+)	+	(+)	+	+

Example 2

Focused Screening

Selected factors from the formulation screening that provided significant effects were further evaluated by combining them in another DoE approach (formulation 1-13; factors: proline, lysine, phosphate, citrate) as well as formulations varying single parameters (Lysine/ succinate (formulation 15); Proline/ succinate (formulation 16); Lysine/ citrate/ poloxamer (formulation 17); Trehalose/ citrate/ lysine (formulation 18)). Formulation 19 displays the prior art formulation corresponding to Enbrel[®] formulation.

Table 7. Formulation 1-19 evaluated during focused screening

[illegible]

Resulting formulations were subjected to a short term stress stability study (Table 8). The analytical methods SEC, CEX and RPC were applied.

Table 8. Analytical methods and stress conditions applied during focused screening

<i>Methods</i>	<i>Stress conditions</i>	<i>Pull points</i>
<i>Stirring (600 rpm)</i>		
SEC	Formulation 1 - 19	48 hours
<i>Freeze / Thaw cycles</i>		
SEC	Formulation 1 - 19	3 cycles
<i>Temperatures</i>		
SEC	Formulation 1 - 13	0 weeks
SEC	Formulation 1 - 13	2 weeks
SEC	Formulation 1 - 13	4 weeks
SEC, CEX, RPC	Formulation 14 - 19	0 weeks
SEC, CEX, RPC	Formulation 14 - 19	2 weeks
SEC, CEX, RPC	Formulation 14 - 19	4 weeks

5

The results of the formulations 1 to 13 are shown in Figure 1 A-D. The factors lysine, proline, citrate buffer and phosphate buffer were evaluated regarding the formation of degradation products due to the storage of TNFR:Fc formulations at 25 (A) and 40°C (B), as well as due to applied stress during stirring for 48 h (C) and three freezing and thawing cycles (D). A positive influence on the quality attributes of TNFR:Fc for the out parameters SEC was defined as:

10

- Sum DPs (SEC) – low level, no increase

A significantly positive effect was determined for the factor citrate buffer, which led to low degradation product levels in the reulating TNFR:Fc formulations at the in table 8 described conditions. Besides that only proline showed a slightly significant effect at storage at 25°C and after 48h of stirring at 600 rpm, where degradation products were increasing with the applied stress.

15

The evaluation of the excipients succinate buffer, in combination with lysine or proline, poloxamer and trehalose were evaluated via RPC, SEC and CEX analysis (Table 9). A positive influence on the quality attributes of TNFR:Fc was defined as:

20

- Content (RPC) –no decrease
- Sum APs (CEX) – low level
- Sum DPs (SEC) – low level

The formulations containing succinate (formulation 15 (including lysine), formulation 16 (including proline)) showed the formation of additional degradation peaks at 40°C storage. Thus, succinate was excluded from further evaluations. As the surfactant

25

poloxamer and the stabilizer trehalose did not show a superior impact on stability (in the range of the prior art formulation corresponding to Enbrel®), poloxamer and trehalose were also excluded from further evaluations.

- 5 Table 9. Summary of influences of excipients succinate, poloxamer and trehalose evaluated during focused screening.

	Succinate		Poloxamer	Trehalose	Target
	Lysin	Proline			
Content (RPC)	Decrease	Decrease	No decrease	No decrease	No decrease
DPs (SEC)	Increase	Increase	Low level	Low level	Low level
APs (CEX)	Increase	Increase	Low level	Low level	Low level

Example 3

Formulation optimization

- 10 The most promising four formulations (formulation 1 – 4) from prior evaluations were subjected to a long term stability study at intended (2-8°C) as well as accelerated (25 and 40°C) storage conditions. The long term stability study was performed with TNFR:Fc formulation filled in syringes (0,5 ml) and vials (1 ml). Table 10 gives an overview on the tested formulations. As reference served the prior art formulation
- 15 corresponding to Enbrel® (formulation 5). All formulation were compared to each other as well as to the described reference.

Table 10. Overview tested formulations formulation optimization

Batch	Strength [mg/ml]	Composition	Fill volume [ml]	Container
1030ML220_1_s	50	50 mM citrate, 29 mM sucrose, 25 mM NaCl, 25 mM L-Lysine, pH 6.3	0.5	syringe
1030ML220_2_s	50	25 mM citrate, 29 mM sucrose, 63 mM NaCl, 25 mM L-Lysine, pH 6.3	0.5	syringe
1030ML220_3_s	50	25 mM citrate, 29 mM sucrose, 75 mM NaCl, 25 mM L-Proline, pH 6.3	0.5	syringe
1030ML220_4_s	50	25 mM phosphate, 29 mM sucrose, 75 mM NaCl, 25 mM L-Lysine, pH 6.3	0.5	syringe
1030ML220_5_s	50	25 mM phosphate, 29 mM sucrose, 75 mM NaCl, 25 mM L-Arginine, pH 6.3	0.5	syringe
1030ML220_1_v	50	50 mM citrate, 29 mM sucrose, 25 mM NaCl, 25 mM L-Lysine, pH 6.3	1.0	2R vial
1030ML220_2_v	50	25 mM citrate, 29 mM sucrose, 63 mM NaCl, 25 mM L-Lysine, pH 6.3	1.0	2R vial

1030ML220_3_v	50	25 mM citrate, 29 mM sucrose, 75 mM NaCl, 25 mM L-Proline, pH 6.3	1.0	2R vial
1030ML220_4_v	50	25 mM phosphate, 29 mM sucrose, 75 mM NaCl, 25 mM L-Lysine, pH 6.3	1.0	2R vial
1030ML220_5_v	50	25 mM phosphate, 29 mM sucrose, 75 mM NaCl, 25 mM L-Arginine, pH 6.3	1.0	2R vial

A positive effect for the evaluated factors lysine and proline as stabilizer as well as citrate and phosphate buffers systems was declared if the stability for output parameters summation of post peak (RPC), summation of aggregation and degradation products (SEC) as well as summation of acid peaks (CEX) was exceeded compared to the reference. The targets for output parameters are described in Table 11.

Table 11. Target description during formulation optimization

Output parameter	Method	Target
Summation of post peaks	RPC	Low level
Summation of aggregation products	SEC	Low level
Summation of degradation products	SEC	Low level
Summation of acidic peaks	CEX	Low level

Results for the output parameters RPC / summation of post peak (PP), SEC / summation of aggregation (APs) and degradation products (DPs) and CEX / summation of acid peaks (APs) are displayed in Table 12, 13 (storage at 2-8°C), Table 14, 15 (storage at 25°C) as well as Table 16, 17 (storage at 40°C). Analysis of samples was performed at start of stability study as well as after 1, 2 and 3 month storage at the declared temperatures.

Result summary for TNFR:Fc formulation stored at 2-8, 25 and 40°C for three month (Table 12)

1) RPC – Summation of post peaks (Sum PPs)

After three month storage at 2-8, 25 and 40°C, TNFR:Fc formulations containing a citrate buffer system (formulation 1-3) displayed lower values of post peaks compared to formulations containing a phosphate buffer (formulation 4 and 5). The absolute lowest value was detected for the formulation containing 50mM citrate buffer and 25 mM lysine (formulation 1). The described effect was even more pronounced for TNFR:Fc formulations stored in vials.

2) SEC – Summation of aggregation products (Sum APs)

After 3 month storage at 2-8, 25 and 40°C, TNFR:Fc formulation 1 to 5 are comparable regarding the amount of aggregation products. In addition no difference was detected between of formulation filled in vials or syringes.

3) SEC – Summation of degradation products (Sum DPs)

After three month storage at 2-8, 25 and 40°C TNFR:Fc formulations containing a citrate buffer system (formulation 1-3) displayed significantly lower values of degradation products compared to formulations containing a phosphate buffer (formulation 4 and 5). The absolute lowest value was detected for the formulation containing 50mM citrate buffer and 25 mM lysine (formulation 1). The described effect was even more pronounced for TNFR:Fc formulations stored in vials.

4) CEX – Summation of acidic peaks (Sum APs)

After three month storage at 2-8, 25 °C and 40°C TNFR:Fc formulations in pre-filled syringes, containing a citrate buffer system (formulation 1-3) displayed significantly lower values of acidic peaks compared to formulations containing a phosphate buffer (formulation 4 and 5). The described effect was more pronounced for TNFR:Fc formulations stored at 2-8°C in vials.

Conclusion formulation optimization

Stability of formulations 1 to 5 during three month storage at 2-8, 25 and 40°C, was assessed via RPC, SEC and CEX analysis. Formulations containing a citrate buffer system (formulation 1- 3) were determined to be superior compared to formulations containing a phosphate buffer system (formulation 4 and 5) regarding the formation of post peaks (RPC), the formation of degradation products (SEC) and the formation of acid peaks (CEX). The absolute best results were determined via RPC and SEC for the formulation containing a 50 mM citrate buffer system and 25 mM lysine as stabilizer. Among the worst, if not the worst formulation was the reference, prior art formulation corresponding to Enbrel® (formulation 5). The degradation of TNFR:Fc, determined via RPC, SEC and CEX was in general more pronounced if formulations were stored in vials. This is most probably due to the greater liquid/air interaction surface displayed by vials compared to syringes. The effects were therefore partially even more pronounced, including the superior stabilization of TNFR:Fc formulated in the citrate / lysine formulation matrix.

Table 12. Summary results of formulation optimization

	RPC		SEC		CEX
	Sum PPs	Sum APs	Sum DPs	Sum APs	
2-8°C	Formulations containing citrate display significantly lower values than the ones containing phosphate; Vials: effect more pronounced; Best formulation: 50mM Citrate/25mM Lysine;	Formulation 1 to 5 are comaparable	Formulations containing citrate display significantly lower values than the ones containing phosphate; Vials: effect more pronounced; Best formulation: 50mM Citrate/25mM Lysine;	Formulations containing citrate display significantly lower values than the ones containing phosphate; Vials: effect more pronounced;	
25°C	See 2-8°C	See 2-8°C	See 2-8°C	Formulations containing citrate display significantly lower values than the ones containing phosphate;	
40°C	See 2-8°C	See 2-8°C	See 2-8°C	See 25°C	

TNFR:Fc stored at 2-8°C – syringes and vials

Table 13. Syringes – 2-8°C

RPC Sum PPs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	10,2	10,2	10,2	10,3	10,3
3M	11,2	11,6	11,7	12,5	12,7

SEC Sum APs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	0,7	0,8	0,7	0,7	0,7
1M	0,7	0,7	0,7	0,6	0,7
2M	0,8	0,8	0,8	0,8	0,8
3M	0,7	0,8	0,8	0,8	0,8

SEC Sum DPs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	4,0	4,1	3,9	4,4	4,4
1M	4,8	4,8	4,9	7,4	7,5
2M	6,0	6,2	6,1	10,3	10,0
3M	6,0	6,5	6,5	12,8	12,8

CEX Sum APs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	9,3	8,3	9,1	8,2	9,2
3M	10,0	10,5	10,8	12,7	13,1

Table 14. Vials – 2-8°C

RPC Sum PPs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	10,1	10,2	10,2	10,3	10,3
3M	11,5	11,9	12,1	13,1	13,0

SEC Sum APs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	0,7	0,7	0,7	0,6	0,7
1M	0,6	0,6	0,6	0,5	0,6
2M	0,7	0,7	0,7	0,7	0,7
3M	0,8	0,8	0,8	0,7	0,7

SEC Sum DPs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	4,0	4,1	4,3	4,7	4,9
1M	6,2	6,3	6,5	8,8	9,0
2M	6,5	7,7	8,8	12,9	13,5
3M	6,1	9,6	9,9	17,1	17,4

CEX Sum APs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	8,2	9,0	8,3	9,1	8,8
3M	11,2	11,5	12,5	13,4	14,3

5 *TNFR:Fc stored at 25°C – syringes and vials*

Table 15. Syringes – 25°C

RPC Sum PPs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	10,2	10,2	10,2	10,3	10,3
1M	11,1	11,4	11,4	12,2	12,4
2M	11,9	12,3	12,3	14,0	14,0
3M	13,3	13,9	13,7	16,4	16,1

SEC Sum APs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	0,0	0,0	0,0	0,0	0,0
1M	1,0	1,0	1,0	1,0	1,0
2M	1,7	1,7	1,7	1,7	1,7
3M	1,9	2,0	2,0	2,0	1,9

SEC Sum DPs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	0,0	0,0	0,0	0,0	0,0
1M	8,3	8,7	8,3	13,4	12,8
2M	9,8	10,8	10,7	18,1	17,6
3M	11,6	12,8	12,6	21,3	20,7
CEX Sum APs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	0,0	0,0	0,0	0,0	0,0
1M	10,0	10,7	10,4	12,6	12,8
2M	12,7	13,1	12,9	15,2	15,3
3M	12,9	13,4	13,5	17,8	18,5

Table 16. Vials – 25°C

RPC Sum PPs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	10,1	10,2	10,2	10,3	10,3
1M	11,4	11,9	11,8	11,7	14,1
2M	12,0	12,8	12,9	17,4	17,8
3M	13,3	14,1	14,3	19,2	20,7

SEC Sum APs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	0,0	0,0	0,0	0,0	0,0
1M	0,9	1,0	0,9	0,9	0,8
2M	1,6	1,6	1,6	1,4	1,3
3M	1,9	2,0	2,0	1,6	1,5

SEC Sum DPs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	0,0	0,0	0,0	0,0	0,0
1M	8,9	10,1	10,0	23,5	24,3
2M	11,2	12,6	12,4	30,9	32,5
3M	13,3	14,8	14,4	36,5	39,0

CEX Sum APs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	0,0	0,0	0,0	0,0	0,0
1M	10,5	10,5	10,9	10,7	15,8
2M	12,8	13,7	13,7	19,6	19,9
3M	14,3	15,4	12,0	14,2	14,2

TNFR:Fc stored at 40°C – syringes and vials

Table 17. Syringes – 40°C

RPC Sum PPs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	10,2	10,2	10,2	10,3	10,3
1M	12,8	13,2	13,2	14,7	14,8
2M	16,8	16,9	17,0	18,8	18,8
3M	19,6	19,6	19,5	22,5	22,9

SEC Sum APs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	0,7	0,8	0,7	0,7	0,7
1M	3,0	3,0	3,0	3,2	3,2
2M	3,1	3,6	3,6	3,8	3,8
3M	7,9	8,2	7,9	8,2	8,3

SEC Sum DPs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	4,0	4,1	3,9	4,4	4,4
1M	9,8	10,0	9,9	13,3	13,0
2M	14,9	14,7	14,4	19,0	19,5
3M	18,4	18,9	18,8	24,3	24,2

CEX Sum APs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	9,3	8,3	9,1	8,2	9,2
1M	13,6	13,1	13,2	15,1	14,5
2M	18,0	19,2	18,9	20,5	19,9
3M	28,1	27,1	26,3	29,8	30,6

Table 18. Vials – 40°C

RPC Sum PPs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	10,1	10,2	10,2	10,3	10,3
1M	13,3	13,0	13,0	14,9	15,2
2M	15,8	16,2	15,6	18,7	18,5
3M	19,1	20,0	19,6	23,9	24,1

SEC Sum APs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	0,7	0,7	0,7	0,6	0,7
1M	2,9	2,8	2,8	2,9	2,8
2M	3,5	3,7	3,6	3,7	3,7
3M	7,5	7,8	7,5	7,5	7,6

SEC Sum DPs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	4,0	4,1	4,3	4,7	4,9
1M	10,9	11,4	11,1	15,5	15,4
2M	15,1	15,9	15,1	21,4	20,9
3M	19,5	20,3	19,9	27,5	27,6

CEX Sum APs	50mMCitrate/ 25mM Lysine	25 mM Citrate/ 25 mM Lysine	Citrate 25mM/ Proline 25mM	Phosphate 25mM/ Lysin 25mM	Phosphate 25mM/ Arginine 25mM
T0	8,2	9,0	8,3	9,1	8,8
1M	14,1	14,6	13,6	15,6	15,7
2M	19,0	19,3	18,5	20,5	21,7
3M	28,0	26,8	26,9	29,9	32,4

LIST OF REFERENCES

5 WO 03/072060 A2
EP1478394

Baynes et al, Role of arginine in the stabilization of proteins against aggregation. Biochemistry, 2005 Mar 29;44(12):4919-25.

10

Shiraki et al, Biophysical effect of amino acids on the prevention of protein aggregation. J Biochem, 2002 Oct;132(4):591-5.

15

Bolli et al, L-Proline reduces IgG dimer content and enhances the stability of intravenous immunoglobulin (IVIG) solutions. Biologicals, 2010 Jan;38(1):150-7.

Zheng and Janis, Influence of pH, buffer species, and storage temperature on physiochemical stability of a humanized monoclonal antibody LA298. Int J Pharm, 2006 Feb 3;308(1-2):46-51.

20

Kolhe et al, Impact of freezing on pH of buffered solutions and consequences for monoclonal antibody aggregation. Biotechnol Prog, 2010 May-Jun;26(3):727-33.

Claims

1. Pharmaceutical composition, comprising TNFR:Fc, a citrate buffer at a concentration from 25 mM to 120 mM, and an amino acid at a concentration from 15 mM to 100 mM, wherein the amino acid is lysine or a pharmaceutically acceptable salt thereof and/or proline or a pharmaceutically acceptable salt thereof.
5
2. The pharmaceutical composition of claim 1, wherein the amino acid is lysine or a pharmaceutically acceptable salt thereof.
10
3. The pharmaceutical composition of claim 1, wherein the amino acid is proline or a pharmaceutically acceptable salt thereof.
4. The pharmaceutical composition according to any one of claims 1 to 3, further comprising at least one tonicity modifier.
15
5. The pharmaceutical composition of claim 4, wherein the at least one tonicity modifier is sodium chloride, cysteine, histidine, glycine, potassium chloride, sucrose, glucose, mannitol, or any combination thereof.
20
6. The pharmaceutical composition of claim 4, wherein the at least one tonicity modifier is sodium chloride and/or sucrose.
7. The pharmaceutical composition according to any one of claims 4 to 6, comprising the at least one tonicity modifier at a total concentration from 5 mM to 200 mM.
25
8. The pharmaceutical composition according to any one of claims 1 to 7, further comprising at least one excipient.
30
9. The pharmaceutical composition of claim 8, wherein the at least one excipient is lactose, glycerol, xylitol, sorbitol, mannitol, maltose, inositol, trehalose,

glucose, bovine serum albumin (BSA), dextran, polyvinyl acetate (PVA), hydroxypropyl methylcellulose (HPMC), polyethyleneimine (PEI), gelatine, polyvinylpyrrolidone (PVP), hydroxyethylcellulose (HEC), polyethylene glycol (PEG), ethylene glycol, glycerol, dimethylsulfoxide (DMSO),
5 dimethylformamide (DMF), L- serine, sodium glutamate, alanine, glycine, sarcosine, gamma-aminobutyric acid (GABA); polyoxyethylene sorbitan monolaurate; polyoxyethylene sorbitan monooleate; sodium dodecyl sulphate (SDS), polysorbate, polyoxyethylene copolymer, potassium phosphate, sodium acetate, ammonium sulfate, magnesium sulfate, sodium sulfate,
10 trimethylamine N-oxide, betaine, zinc ions, copper ions, calcium ions, manganese ions, magnesium ions, CHAPS, sucrose monolaurate, 2-O-beta-mannoglycerate, or any combination thereof.

10. The pharmaceutical composition of claim 8 or 9, comprising the at least one
15 excipient at a total concentration from 0.1 mM to 0.7 mM.

11. The pharmaceutical composition according to any one of claims 1 to 10, comprising TNFR:Fc at a concentration from 0.1 mM to 0.7 mM.

20 12. The pharmaceutical composition according to any one of claims 1 to 11, wherein said composition further comprises a pharmaceutically acceptable solvent.

13. The pharmaceutical composition according to any one of claims 1 to 11,
25 wherein the composition is lyophilized.

14. The pharmaceutical composition of claim 1,
comprising 0.1 mM to 0.7 mM TNFR:Fc, 25 mM to 120 mM citrate buffer, 15 mM to 100 mM lysine, 10 to 100 mM sucrose and 5 mM to 200 mM sodium
30 chloride at a pH value of about 6.3; or
comprising 0.3 mM TNFR:Fc, 50 mM citrate buffer, 25 mM lysine, 29 mM sucrose and 75 mM sodium chloride at a pH value of about 6.3; or

comprising 0.3 mM TNFR:Fc, 25 mM citrate buffer, 25 mM lysine, 29 mM sucrose and 88 mM sodium chloride at a pH value of about 6.3; or
comprising 0.1 mM to 0.7 mM TNFR:Fc, 25 mM to 120 mM citrate buffer, 15 mM to 100 mM proline, 10 mM to 100 mM sucrose and 5 mM to 200 mM sodium chloride at a pH value of about 6.3; or
comprising 0.3 mM TNFR:Fc, 25 mM citrate buffer, 25 mM proline, 29 mM sucrose and 75 mM sodium chloride at a pH value of about 6.3.

15. The pharmaceutical composition according to any one of claims 1 to 14, wherein TNFR:Fc is etanercept.
16. Kit comprising a composition according to any one of claims 1 to 15 and instructions for use of said composition.
17. The kit of claim 16, wherein the composition is contained in a pre-filled syringe or wherein the composition is contained in a pre-filled vial.
18. The kit of claim 16, wherein the composition is lyophilized and contained in a vial.
19. Method of producing a pharmaceutical composition according to any one of claims 1 to 15, comprising combining TNFR:Fc, a citrate buffer at a concentration from 25 mM to 120 mM and an amino acid at a concentration from 15 mM to 100 mM, wherein the amino acid is lysine or a pharmaceutically acceptable salt thereof and/or proline or a pharmaceutically acceptable salt thereof.
20. The method of claim 19, wherein the amino acid is lysine or a pharmaceutically acceptable salt thereof.
21. The method of claim 19, wherein the amino acid is proline or a pharmaceutically acceptable salt thereof.

22. The method of any one of claims 19 to 21, further comprising adding at least one tonicity modifier, and/or a pharmaceutically acceptable solvent.
23. The method of claim 22, further comprising adding at least one excipient as
5 defined in any one of claims 8 to 10.
24. The method according to any one of claims 19 to 23, further comprising a lyophilization step.
- 10 25. Composition of any one of claims 1 to 15 for use in treatment of diseases, wherein the diseases are autoimmune disease, ankylosing spondylitis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis, rheumatoid arthritis, Wegener's disease (granulomatosis), Crohn's disease or inflammatory bowel disease, chronic obstructive pulmonary disease (COPD), Hepatitis C,
15 endometriosis, asthma, cachexia, atopic dermatitis, Alzheimer, cancer, or any combination thereof.
26. Use of the composition of any one of claims 1 to 15 for treatment of diseases, wherein the diseases are autoimmune disease, ankylosing spondylitis,
20 juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis, rheumatoid arthritis, Wegener's disease (granulomatosis), Crohn's disease or inflammatory bowel disease, chronic obstructive pulmonary disease (COPD), Hepatitis C, endometriosis, asthma, cachexia, atopic dermatitis, Alzheimer, cancer, or any combination thereof.
- 25 27. Use of the composition of any one of claims 1 to 15 for the preparation of a medicament for treatment of diseases, wherein the diseases are autoimmune disease, ankylosing spondylitis, juvenile rheumatoid arthritis, psoriasis, psoriatic arthritis, rheumatoid arthritis, Wegener's disease (granulomatosis),
30 Crohn's disease or inflammatory bowel disease, chronic obstructive pulmonary disease (COPD), Hepatitis C, endometriosis, asthma, cachexia, atopic dermatitis, Alzheimer, cancer, or any combination thereof.