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(54) Title: STABLE PHARMACEUTICAL COMPOSITION OF TNFR:FC FUSION PROTEIN

(57) Abstract: The present invention relates to the stable pharmaceutical compositions comprising tumor necrosis factor receptor Fc fusion protein (TNFR:Fc). More particularly, it relates to the stable pharmaceutical compositions comprising tumor necrosis factor receptor Fc fusion protein (TNFR:Fc), phosphate - citrate buffer. It also relates to the methods of manufacturing the composition, method of administration and kits containing the same.

STABLE PHARMACEUTICAL COMPOSITION OF TNFR:FC FUSION PROTEIN

Field of Invention

The invention provides stable pharmaceutical compositions comprising tumor necrosis factor receptor Fc fusion protein (TNFR:Fc). The invention also provides methods of manufacturing the composition, method of administration and kits containing the same.

Background of Invention

Tumor necrosis factor (TNF) alpha is a cytokine that promotes the inflammation and its associated signs by binding to its receptor. It is produced by macrophages and many other immune cells. It is involved in pathogenesis of many inflammatory disorders like rheumatoid arthritis, psoritic arthritis, SLE, Crohn's disease etc. Hohmann et al (Hohmann et al. 1989 *J Biol Chem.* 25, 14927-34) identified 2 distinct receptors of TNF-alpha which are present on different cell types viz. myeloid cells and epithelial cells. Using monoclonal antibodies, Brockhaus et al (Brockhaus et al. 1990 *ProcNatlAcadSci U S A.*, 87(8), 3127-31) demonstrated that both TNF-alpha and beta bind to both the receptors with high affinity.

Tumor necrosis factor-alpha (TNF-alpha) is a central regulator of inflammation, and TNF-alpha antagonists may be effective in treating inflammatory disorders in which TNF-alpha plays an important pathogenetic role. Inhibition of TNF has proven to be an effective therapy for patients with rheumatoid arthritis and other forms of inflammatory disease including psoriasis, psoriatic arthritis, and ankylosing spondylitis, inflammatory bowel disease. One such TNF-alpha antagonist is Etanercept.

Etanercept is a dimeric fusion protein produced by recombinant DNA technology where gene of soluble, ligand binding portion of TNF receptor 2 is fused with gene of Fc component of human IgG1 to give the desired fusion protein (US 7648702). Etanercept is expressed in CHO cells. The Fc component of Etanercept lacks CH1 domain but has CH2, CH3 domains and hinge region. The fusion protein has approximate molecular weight of 150 kD and consists of 934 amino acids. Etanercept interferes with TNF and acts as a TNF inhibitor due to which it can be used as a biopharmaceutical to treat

autoimmune diseases. It prevents progressive destruction of joints in patients with rheumatoid arthritis and the arthritis of psoriasis.

Due to its unique structure, Etanercept binds 50-100 folds more efficiently to TNF alpha than its endogenous receptor (Gofeeet al. 2003*J Am AcadDermatol.* 49, S105-111, Strober 2005 *SeminCutan Med Surg.* 24; 28-36). Additionally, due to its dimeric nature it can bind to 2 TNF alpha molecules as compared to one bound by endogenous receptor. Conjugation of this molecule to Fc region of IgG increases the half life as compared to endogenous soluble form. Commercially, Etanercept is available in both Lyophilized and liquid forms.

The most important feature of a composition is to help the protein to retain its structural conformation or its activity. The stability of protein in a composition can be related with long-term storage. It is understood to mean that the active polypeptide of the pharmaceutical composition does not substantially lose its activity as compared to the composition at the beginning of storage.

All polypeptides have an Isoelectric Point (pI), which is generally defined as the pH at which a polypeptide carries no net charge. It is known in the art that protein solubility is typically lowest when the pH of the solution is equal to the isoelectric point (pI) of the protein.

The Tm of the Fab domain of a protein is a good indicator of the thermal stability of a protein and may further provide an indication of the shelf-life. Tm values of proteins determined by differential scanning calorimetry, give insight into heat-induced changes in protein conformation, mechanisms of protein unfolding and stabilization in solution. A lower Tm indicates less stability of a protein in given solution, whereas a higher Tm indicates a better stability of the protein. The Tm of the protein will vary based on the formulation composition which in turn reflects its stability in respective formulation.

During long term storage, both aqueous and lyophilized compositions of proteins can lose active protein due to aggregation or degradation. Aggregation of the protein can lead to

immunogenicity and is undesirable. Since the concentration of Etanercept used in the composition is high, there is a likely possibility of protein aggregation during long term storage. To improve the stability of the protein either the concentration of the existing excipients can be varied or new excipients can be added to modify the composition.

US Patent Nos US 5,215,743; US 7,648,702; US application US 20070053906 and WO 2011141926 disclose pharmaceutical compositions comprising aqueous composition of TNF-binding protein comprising a TNF-binding protein, a buffer and an isotonicity agent.

Summary of the Invention

In an embodiment, the invention is related to a stable pharmaceutical composition comprising TNFR:Fc fusion protein and phosphate – citrate buffer.

In another embodiment, the invention is related to a stable pharmaceutical composition comprising TNFR:Fc fusion protein, phosphate – citrate buffer and anti-aggregating agent selected from L-glycine, urea and 2-hydroxypropyl beta-cyclodextrin (HPBCD).

In another embodiment, the invention is related to a stable pharmaceutical composition comprising TNFR:Fc fusion protein, phosphate – citrate buffer, anti-aggregating agent selected from L-glycine, urea and HPBCD, a tonicity modifying agent and a stabilizing agent.

In yet another embodiment, the invention is related to the method of treating a disease using the stable pharmaceutical composition of the present invention. The disease may be rheumatoid arthritis, polyarticular juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis or plaque psoriasis.

In another embodiment the invention is related to a kit or container containing the pharmaceutical composition of the invention.

The details of one or more embodiments of the invention set forth below are illustrative

only and not intended to limit to the scope of the invention. Other features, objects and advantages of the inventions will be apparent from the description and claims.

Detail Description of Invention

The invention provides a stable pharmaceutical composition comprising TNFR molecules fused to an Fc portion of a human immunoglobulin (TNFR:Fc fusion protein). More particularly, the invention relates to the stable pharmaceutical composition of etanercept in phosphate - citrate buffer, which displays a lower degradation potential.

In an embodiment of the invention, the TNFR:Fc fusion protein is etanercept.

It has been reported in US 7648702 patent and WO2011141926 application that the pharmaceutical compositions of Etanercept using L-glycine as anti-aggregating agent in phosphate buffer are not stable as compared to the compositions of etanercept with other amino acid such as arginine, proline, lysine, aspartic acid as anti-aggregating agent in phosphate buffer. The WO2011141926 application discloses that the composition comprising Etanercept in phosphate buffer and L-glycine as anti-aggregating agent showed aggregation as well as fragmentation products.

While studying the Etanercept compositions in different buffers and using different anti-aggregating agents it was observed that Etanercept composition comprising phosphate – citrate buffer with L-glycine as anti-aggregating agent showed improved stability as compared to Etanercept composition comprising phosphate buffer with L-glycine as anti-aggregating agent at 5 °C and 40 °C.

As illustrated in the example section, the stability of Etanercept composition essentially consisting of phosphate – citrate buffer in combination with L-glycine as anti-aggregating agent were assessed during a 6 months stability study at 5 °C as well as 2 weeks study at 40 °C (stress conditions stability studies). Compositions comprising the phosphate – citrate buffer system were determined to be superior as compared to composition comprising phosphate buffer system with respect to the % aggregation products and & degradation products as determined by SEC.

The stable pharmaceutical composition used herein means that the TNFR:Fc fusion protein exhibits following features:

i. The stable pharmaceutical composition of TNFR:Fc fusion protein in phosphate – citrate buffer exhibits improved stability as compared to the composition of etanercept comprising phosphate buffer, arginine and sodium chloride. The % aggregates are less in the Etanercept composition comprising L-glycine as anti-aggregating agent in phosphate-citrate buffer as determined after 2 weeks of storage at 40°C by SEC.

ii. The stable pharmaceutical composition of TNFR:Fc fusion protein in phosphate – citrate buffer exhibits less than 5% high and low molecular weight impurities similar to the innovator composition of etanercept comprising phosphate buffer, arginine and sodium chloride after 2 weeks of storage at 40°C by SEC.

iii. The stable pharmaceutical composition of TNFR:Fc fusion protein in phosphate – citrate buffer, glycine exhibits approximately 8% high and low molecular weight impurities. Whereas the composition of etanercept comprising phosphate buffer, glycine as an anti-aggregating agent showed ~ 15% impurities, after 2 weeks of storage at 40°C by SEC.

In another embodiment the invention relates to the pharmaceutical composition of Etanercept in phosphate-citrate buffer with other anti-aggregating agents such as urea, HPBCD.

After obtaining improved stability of Etanercept composition in phosphate –citrate buffer other anti-aggregating agents from other class of compounds than amino acids were tested.

It was observed that Urea and HPBCD also provided stable pharmaceutical compositions of Etanercept in phosphate – citrate buffer. It is understood to mean that etanercept of the pharmaceutical composition does not substantially lose its activity as compared to the composition at the beginning of storage. The term 'substantially' refers to not more than

20%, or more preferably 15%, or even more preferably 10%, and most preferably 5% of its activity relative to activity of the composition at the beginning of storage. The pharmaceutical composition of the invention is suitable for long term storage. As used herein, 'the long term storage' means that the storage of the pharmaceutical composition is stable for more than a month, preferably more than 6 months or 12 months, more preferably more than 24 months.

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 tumorgenesis and viral replication. Dysregulation of TNF-alpha production has been implicated in a variety of human diseases like 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 as well as cancer.

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.

In an embodiment the TNFR:Fc may be administered in adult or juvenile subject, wherein the amount may range from about 1 – 80 mg. The dose may be administered once weekly, twice weekly. Further, the doses may be administered weekly, biweekly, or separated by several weeks e.g. three weeks. The therapeutic dose and duration may vary as per patient response and patient requirement.

In another embodiment, a suitable regimen for juvenile and paediatric patients may involve a dose of 0.4 mg/kg to 5 mg/kg of TNFR:Fc, administered one or more times per week.

In case of adult rheumatoid arthritis, 25 mg twice weekly or 50 mg once weekly TNFR:Fc may be administered.

In case of psoriatic arthiritis, 25 mg twice weekly or 50 mg once weekly TNFR:Fc may be administered.

In case of Ankolysing spondylitis, 25 mg twice weekly or 50 mg once weekly TNFR:Fc may be administered.

In case of adult plaque psoriasis, the recommended dose of TNFR:Fc is 25 mg administered twice weekly or 50 mg administered once weekly. In case of pediatric plaque psoriasis, the recommended dose of TNFR:Fc is 0.8 mg/Kg weekly with a maximum of 50 mg dose per week.

In case of polyarticular juvenile idiopathic arthritis, the recommended dose of TNFR:Fc is 0.8 mg/Kg weekly with a maximum of 50 mg dose per week.
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.

Any suitable syringe or vial or cartridge 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 composition 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 composition 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.

The pharmaceutical composition is sterile and stable for long period of time at 2-8^0 C. Also it is stable upto 6 months when stored at 25^{0}C. The invention provides pharmaceutical composition essentially comprising of etanercept, phosphate – citrate buffer, anti-aggregating agent selected from L-glycine, urea and HPBCD, a tonicity modifier, a stabilizer and optionally other excipients in suitable combination thereof.

The invention further relates to a stable pharmaceutical composition, wherein the composition is liquid or lyophilized. The invention is further related to a stable pharmaceutical composition in a pre-filled syringe, vial, cartridge, or pen.

In an embodiment of the invention, the active pharmaceutical ingredient etanercept is used which is obtained from recombinant DNA technology using CHO cells. The concentration of the etanercept in the composition is 10 mg/mL to 100 mg/mL. In a preferred embodiment of the invention, the concentration of etanercept in the composition is 10 mg/mL to 60 mg/mL. In the most preferred embodiment of the invention, the concentration of etanercept in the composition is 20 mg/mL to 60 mg/mL.

In another embodiment of the invention, the buffer is phosphate – citrate buffer. In an embodiment of the invention, the concentration of the buffer in the composition is 10 mM to 100 mM. In a preferred embodiment of the invention, the concentration of the buffer in the composition is 10 mM to 50 mM. In another preferred embodiment of the invention, the concentration of the buffer in the composition is 20 mM to 40 mM.

In another embodiment of the invention, the pH of the composition is 5 to 8.
In another embodiment of the invention, the etanercept composition comprises anti-aggregating agent selected from L-glycine, urea and HPBCD.

In an embodiment of the invention when the anti-aggregating agent is L-glycine, then the concentration of L-glycine in the composition is 10 mM to 300 mM.

In another embodiment of the invention when the anti-aggregating agent is urea, the concentration of urea in the composition is 20 mM to 50mM.

In another embodiment of the invention when the anti-aggregating agent is HPBCD, then the concentration of HPBCD is 10 mM to 100mM.

In another embodiment of the invention, the stable pharmaceutical composition further comprises a parenterally acceptable tonicity agent. The tonicity agent is selected from the group of salts such as sodium chloride, potassium chloride, calcium chloride or saccharides such as mannitol, sucrose, glucose, or amino acids such as arginine, cysteine, histidine and the like. The preferred tonicity agent is sodium chloride. The concentration range varies from 0 mM to 150 mM.

In yet another embodiment of the invention, the stable pharmaceutical composition further comprises a stabilizer. The stabilizer is selected from the group consisting of sucrose, trehalose, lactose, mannitol.The preferred stabilizing agent is sucrose. The concentration of the stabilizing agent in the composition varies from 0.5 wt% to 10 wt%. In the most preferred embodiment of the invention, the concentration of the stabilizer in the composition is 0.5 wt% to 1.5 wt%.

In yet another embodiment of the invention, the stable pharmaceutical composition may optionally comprise a chelating agent. The chelating agent is selected from the group consisting of EDTA, DTPA, HEDTA, NTA and TSP. The preferred chelating agent is EDTA. In a more preferred embodiment of the invention, the concentration of EDTA is 0 mM to 10 mM.

In another embodiment of the invention, the stable pharmaceutical composition of the invention comprises stable etanercept, phosphate - citrate buffer; anti-aggregating agent selected from L-glycine, urea or HPBCD; sucrose as a stabilizing agent and with a long shelf life at temperature 5ºC .

In another embodiment of the invention, the stable pharmaceutical composition of the invention comprises stable etanercept, phosphate - citrate buffer; anti-aggregating agent selected from L-glycine, urea or HPBCD; sucrose as a stabilizing agent and with a long shelf life at 5ºC.

In another embodiment of the invention, the stable pharmaceutical composition of the invention comprises stable etanercept, phosphate - citrate buffer; anti-aggregating agent selected from L-glycine, urea or HPBCD; sucrose as a stabilizing agent and with 2 weeks shelf life at 40°C.

In another embodiment of the invention, the stable pharmaceutical composition of the invention comprises stable etanercept, phosphate - citrate buffer; anti-aggregating agent selected from L-glycine, urea or HPBCD; sucrose as a stabilizing agent which provides better stability to the pharmaceutical composition to maintain its activity for the longer period of time providing longer shelf life.

In another embodiment, the invention pertains to a method of producing a pharmaceutical composition according to the first aspect, comprising TNFR:Fc, phosphate - citrate buffer, stabilizing agent selected from the group consisting of L-glycine, urea and HPBCD.

In a preferred embodiment, the method may further comprise the step of adding at least one tonicity modifier, such as sodium chloride; a stabilizer, such as sucrose and optionally a chelating agent as defined above.

In another embodiment, the method may further comprise a lyophilization step, which may be before or after adding the at least one tonicity modifier, and/or an excipient as defined above.

Accordingly to a preferred embodiment of the present invention the pharmaceutical composition comprises 10 mg/ml to 100 mg/ml of etanercept, about 10 mM to 100 mM of phosphate citrate buffer, about 10 mM to 300 mM L-glycine, about 0 mM to 150 mM sodium chloride and about 0.5 wt% to 10wt% sucrose having a pH range of 5 to 7.

Accordingly to another preferred embodiment of the present invention the pharmaceutical composition comprises 10 mg/ml to 100 mg/ml of etanercept, about 10 mM to 100 mM of phosphate citrate buffer, about 1 mg/ml to 18 mg/ml urea, about 1 mM to 150 mM

sodium chloride, about 0.5 wt% to 2 wt% sucrose and about 0mM to 10 mM EDTAhaving a pH range of 5 to 7..

In another preferred embodiment of the present invention the pharmaceutical composition comprises 10 mg/ml to 100 mg/ml of etanercept, about 10 mM to 100 mM of phosphate citrate buffer, about 20 mg/ml to 30 mg/ml HPBCD, about 1 mM to 150 mM sodium chloride, about 0.5 wt% to 2 wt% sucrose and about 0 mM to 10 mM EDTAhaving a pH range of 5 to 7..

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.

Experimental Section

The active ingredient etanercept, which was used for the described examples, is derived from recombinant DNA technology in CHO cells. The CHO cells were cultured in a fed-batch process. Etanercept was purified from the cell free harvest by standard purification and filtration process including affinity chromatography and further chromatographic and filtration steps. Etanercept was derived from different production batches which was used in the examples, i.e., example 2 and example 5.

General Process for Preparation of stable pharmaceutical composition of Etanercept

The process for preparing theEtanercept drug substance compositions comprises of 2 steps viz. preparation of formulated bulk and fill finish. The formulated bulk is prepared by diluting the drug substance with the formulation buffer to achieve the desired concentration of drug product. The formulation buffer is prepared by adding required quantity of Trisodium Citrate dihydrate and Sodium dihydrogen phosphate dihydrate to WFI followed by mixing. Further, required quantities of other excipients are added to the above solution and the desired volume is adjusted with WFI after adjustment of pH. The formulation buffer is then aseptically filtered using 0.22 μ sterilizing grade PVDF filter.

As per the batch calculation, the required quantity of the Etanercept (in same formulation) is aseptically diluted

The compositions were analysed by Size Exclusion - High-performance liquid chromatography (SE-HPCL) at different time frames of storage at 5°C, 25°C and 40°C.

SE-HPLC separates the proteins and its related impurities on the basis of their size. Therefore, it is useful to detect aggregation and fragmentation of Etanercept.

The examples which follow are illustrative of the invention and are not intended to be limiting.

Example 1

The process for preparing theEtanercept drug substance compositions was comprises of 2 steps viz. preparation of formulated bulk and fill finish. The formulated bulk is prepared by diluting the drug substance with the formulation buffer to achieve the desired concentration of drug product. The formulation buffer is prepared by adding required quantity (as mentioned in table 1) of Trisodium Citrate dihydrate and Sodium dihydrogen phosphate dihydrate to WFI followed by mixing. Further, required quantities of Glycine as anti-aggregating agent and other excipients are added to the above solution and the desired volume is adjusted with WFI after adjustment of pH. The formulation buffer is then aseptically filtered using 0.22 µ sterilizing grade PVDF filter. As per the batch calculation, the required quantity of the Etanercept DS (in same formulation) is aseptically diluted with the filtered formulation buffer to achieve the desired concentration of 50 ± 5 mg/mL of Etanercept bulk. The formulated bulk is filtered through 0.22 µ sterilizing grade PVDF filter and is aseptically dispensed into prefilled syringes. The PFSs were then charged on stability at various temperatures.Table 1 describes the composition obtained using L-glycine as anti-aggregating agent in 75 mM concentration.

Table 1: The composition of Example 1

Excipients	Concentration	Molar Concentration
Etanercept	50mg/mL	50mg/mL
NaH2PO4 dihydrate	2.6 mg/mL	16.6 mM
Trisodium Citrate dihydrate	4.5 mg/mL	15.3 mM
Sucrose	10 mg/mL	1%
NaCl	3.8 mg/mL	65 mM
L-glycine	5.6 mg/mL	75 mM

Example 2:

Etanercept compositions were studied in different buffers and using different anti-aggregating agents. The short term stability of Etanercept composition comprising phosphate – citrate buffer with L-glycine as anti-aggregating agent was analysed at 5 °C for 6 months and 40 °C for 2 weeks along with the Etanercept composition comprising phosphate buffer with L-glycine as anti-aggregating agent, where other excipients were maintained constant by using SE-HPLC and the results are provided in Table 2. The Table 2 illustrates the stability studies of different etanercept compositions.

Table 2: Stability studies at 5 °C and 40 °C

	Etanercept in phosphate citrate buffer with L-glycine	**Etanercept in phosphate buffer withL-glycine**
% purity on day 1 @ 5°C	92.7	91.2
% purity after 6 months @ 5°C	85.6	68.2
% purity on day 1 @ 40°C	90.9	89.3
% purity after 2 weeks @ 40°C	82.8	74.9

As table 2 illustrates, Etanercept composition comprising phosphate – citrate buffer with L-glycine as anti-aggregating agent showed improved stability as compared to Etanercept composition comprising phosphate buffer with L-glycine as anti-aggregating agent at 5 °C and 40 °C.

Example 3

The process for preparing the Etanercept drug substance composition is similar as explained in example 1, wherein the anti-aggregating used is urea. Composition shown in Table 3 was prepared using urea as anti-aggregating agent.

Table 3: The composition of Example 3

Excipients	Concentration	Molar Concentration
Etanercept	50mg/mL	50mg/mL
NaH2PO4	2.6 mg/mL	16.6 mM
Trisodium Citrate	4.5 mg/mL	15.3 mM
Sucrose	10 mg/mL	1%
NaCl	5.8 mg/mL	100 mM
EDTA	1.8 mg/mL	5 mM
Urea	1.5 mg/mL	25 mM

Example 4

The process for preparing the Etanercept drug substance composition is similar as explained in example 1, wherein the anti-aggregating used is HPBCD. Composition shown in Table 4 was prepared using HPBCD as anti-aggregating agent.

Table 4: The composition of Example 4

Excipients	Concentration	Molar Concentration
Etanercept	50mg/mL	50mg/mL
NaH2PO4	2.6 mg/mL	16.6 mM
Trisodium Citrate	4.5 mg/mL	15.3 mM
Sucrose	10 mg/mL	1%
NaCl	5.8 mg/mL	100 mM
EDTA	1.8 mg/mL	5 mM
HPBCD	25 mg/mL	17.8 mM

Example 5

The innovator composition of etanerceptcomprising phosphate buffer and arginine as anti-aggregating agent (Composition 1) and the composition of etanercept comprising phosphate – citrate buffer and L-glycine as anti-aggregating agent (Composition 2) were filled in PFSs and were charged on long term stability which is ongoing. The data of

protein purity after 9 months storage at 5°C, 6 months storage at 25°C, 2 weeks storage at 40°C was analysed by using SE-HPCL and the results are provided in table 5.

Table 5: Comparative stability data

	Composition 1	**Composition 2**
% purity on day 0	97.5	97.6
% purity after 9 months @ 5°C	96.9	96.9
% purity after 6 months @ 25°C	94.1	93.5
% purity after 2 weeks @ 40°C	95.3	95.1

Similarly, the compositions of example 3 and example 4, i.e., composition of etanercept comprising phosphate – citrate buffer with urea as anti-aggregating agent and composition of etanercept comprising phosphate – citrate buffer with HPBCD as anti-aggregating agent were filled in PFSs and were studied for stability at 5°, 25° and 40°C. The stability of these compositions was assessed and was found comparable with the composition 1 upto period of 2 weeks.

Example 6

Etanercept used for the lyophilization studies is formulated and dialyzed extensively with pharmaceutical compositions mentioned in table 6. Respective formulated bulks are filled in vials, half stoppered and are subjected to lyophilization.

Table 6: The compositions of Example 6

Excipients	**Composition 3**	**Composition 4**
Etanercept	50mg/mL	50mg/mL
NaH2PO4 dihydrate	2.6 mg/mL	2.6 mg/mL
Trisodium Citrate dihydrate	4.5 mg/mL	4.5 mg/mL
Sucrose	3 mg/mL	1 mg/mL
L-glycine	11.24 mg/mL	22.48 mg/mL

All patents, patent applications and publications cited in this application are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual patent, patent application or publication were so individually denoted.

Although certain embodiments and examples have been described in detail above, those having ordinary skill in the art will clearly understand that many modifications are possible in the embodiments and examples without departing from the teachings thereof.

CLAIMS

1. A stable pharmaceutical composition comprising TNFR:Fc fusion protein and phosphate – citrate buffer,.
2. A stable pharmaceutical composition comprising TNFR:Fc fusion protein, phosphate – citrate buffer and anti-aggregating agent selected from the group consisting of L-glycine, urea and 2-hydroxy propyl beta-cyclodextrin (HPBCD).
3. The composition as claimed in claim 1or 2, wherein the pH of the composition is in the range of 5 to 7.
4. The composition of any of the preceding claims wherein TNFR:Fc fusion protein is Etanercept.
5. A stable pharmaceutical composition comprising TNFR:Fc fusion protein, phosphate – citrate buffer, an anti-aggregating agent selected from L-glycine, urea and 2-hydroxy propyl beta-cyclodextrin (HPBCD), a tonicity agent and a stabilizing agent.
6. The composition of claim 5, wherein the tonicity agent is selected from a group of salts consisting of sodium chloride, potassium chloride, calcium chloride; group of saccharides consisting mannitol, sucrose, glucose and amino acids.
7. The composition of claim 5, wherein the stabilizing agent is selected from the group consisting of sucrose and trehalose.
8. The stable pharmaceutical composition of claim 5 comprising of etanercept, phosphate-citrate buffer; L-glycine as anti-aggregating agent; sodium chloride as a tonicity agent and sucrose as a stabilizing agent.
9. The stable pharmaceutical composition of claim 5 comprising etanercept, phosphate-citrate buffer, urea as an anti-aggregating agent, sodium chloride as a tonicity agent, sucrose as a stabilizing agent and EDTA as a chelating agent.
10. The stable pharmaceutical composition of claim 5 comprising etanercept, phosphate-citrate buffer, HPBCD as an anti-aggregating agent, sodium chloride as a tonicity agent, sucrose as a stabilizing agent and EDTA as a chelating agent.
11. The composition of any of the preceding claims wherein the composition is sterile and ready for parenteral administration.
12. The composition of claim 11, wherein the composition is liquid.

13. The composition of claim 11, wherein the composition is lyophilized.
14. The pharmaceutical composition of claim 8, comprising 10 mg/ml to 100 mg/ml of etanercept, about 10 mM to 100 mM of phosphate citrate buffer, about 10 mM to 300 mM L-glycine, about 0 mM to 150 mM sodium chloride and about 0.5 wt% to 10wt% sucrose.
15. The pharmaceutical composition of claim 9, comprising 10 mg/ml to 100 mg/ml of etanercept, about 10 mM to 100 mM of phosphate citrate buffer, about 1 mg/ml to 18 mg/ml urea, about 1 mM to 150 mM sodium chloride, about 0.5 wt% to 2 wt% sucrose and about 0mM to 10 mM EDTA.
16. The pharmaceutical composition of claim 10, comprising 10 mg/ml to 100 mg/ml of etanercept, about 10 mM to 100 mM of phosphate citrate buffer, about 20 mg/ml to 30 mg/ml HPBCD, about 1 mM to 150 mM sodium chloride, about 0.5 wt% to 2 wt% sucrose and about 0 mM to 10 mM EDTA.
17. A kit comprising a composition of any of the preceding claims and instructions for use of the said composition.
18. The Kit of claim 17, wherein the composition is liquid or lyophilized powder.
19. The kit of claim 17, wherein the composition is stored in a pre-filled sterile syringe or vial or cartridge.
20. A method of treating a mammal in need there of comprising administering a therapeutically effective amount of the pharmaceutical composition of any of claims 1 to 16.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2013/059612

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/17 A61K39/395 A61P43/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, INSPEC, BEILSTEIN Data, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/141926 A2 (INTAS BIOPHARMACEUTICALS LTD [IN]; TUNGA BINITA SHRIVASTAVA [IN]; SHAR) 17 November 2011 (2011-11-17)	1,2,4-8, 11,12, 17,18,20
Y	the whole document claims; examples	1-8, 11-14, 17-20

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 December 2013	19/03/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Orlando, Michele

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2013/059612

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	YOSHIKO KITA ET AL: "Salts and Glycine Increase Reversibility and Decrease Aggregation during Thermal Unfolding of Ribonuclease-A.", BIOSCIENCE, BIOTECHNOLOGY, AND BIOCHEMISTRY, vol. 66, no. 4, 1 January 2002 (2002-01-01), pages 880-882, XP055092739, ISSN: 0916-8451, DOI: 10.1271/bbb.66.880 the whole document ----	1-8, 11-14, 17-20
Y	WO 2012/065072 A2 (ABBOTT BIOTECH LTD; NEU MICHAEL [DE]; TSCHOEPE MARKUS [DE]; WEBER CARS) 18 May 2012 (2012-05-18) the whole document claims; examples ----	1-8, 11-14, 17-20
Y	Anonymous: "Enbrel, INN etanercept", European Medicines Agency , 11 September 2009 (2009-09-11), pages 184-185, XP002717733, Retrieved from the Internet: URL:http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000262/WC500027361.pdf [retrieved on 2013-12-11] the whole document ----	1-8, 11-14, 17-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2013/059612

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

8, 14(completely); 1-7, 11-13, 17-20(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 8, 14(completely); 1-7, 11-13, 17-20(partially)

   A stable pharmaceutical composition comprising TNFR:Fc fusion protein, phosphate - citrate buffer and L-glycine as an anti-aggregating agent.

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2. claims: 9, 15(completely); 1-7, 11-13, 17-20(partially)

   A stable pharmaceutical composition comprising TNFR:Fc fusion protein, phosphate - citrate buffer and urea as an anti-aggregating agent.

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3. claims: 10, 16(completely); 1-7, 11-13, 17-20(partially)

   A stable pharmaceutical composition comprising TNFR:Fc fusion protein, phosphate - citrate buffer and 2-hydroxy propyl beta-cyclodextrin (HPBCD) as an anti-aggregating agent.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2013/059612

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011141926 A2	17-11-2011	CN 102946858 A	27-02-2013
		EP 2568960 A2	20-03-2013
		WO 2011141926 A2	17-11-2011
WO 2012065072 A2	18-05-2012	AU 2011325974 A1	09-05-2013
		CA 2815689 A1	18-05-2012
		CN 103458926 A	18-12-2013
		EP 2637690 A2	18-09-2013
		JP 2013543868 A	09-12-2013
		KR 20130135266 A	10-12-2013
		SG 190069 A1	28-06-2013
		TW 201244736 A	16-11-2012
		US 2012263731 A1	18-10-2012
		WO 2012065072 A2	18-05-2012