However, this formulation is not stable for storage. It was discovered that a liquid formulation of etanercept could be achieved using arginine to stabilize the protein (see US Patent No. 7,648,702). An exemplary liquid formulation consists of 50 mg/mL etanercept, 25 mM phosphate buffer, 25 mM L-arginine hydrochloride, 100 mM NaCl, 1% Sucrose at pH 6.3 in water.

Summary

[0006] Described herein are new and improved formulations of etanercept. In particular, the disclosure refers to pharmaceutical compositions containing etanercept that are stable and can be conveniently stored as a liquid at controlled room temperature (CRT) for extended periods of time, even in the absence of an additional buffering agent. In addition, when the pharmaceutical compositions of the invention are injected into subjects, they also demonstrate significantly reduced injection pain as compared to the commercially available prior art formulation. Thus these pharmaceutical compositions are more convenient and advantageous for patients.

[0007] In another example, there are described methods of formulating pharmaceutical preparations of etanercept at a desired pH but in the absence of additional buffering agent in the final formulation.

[0008] In another example, the disclosure refers to a pharmaceutical composition comprising etanercept, NaCl, arginine, and sucrose, wherein the pharmaceutical composition has essentially no additional buffering agent, and the pH of the composition is between 6.1 and 6.5. In one example, the pharmaceutical composition is capable of maintaining the pH between 6.1 and 6.5 when stored at controlled room temperature (CRT) for 2 weeks. In another example, the etanercept concentration is between 40 mg/mL and 100 mg/mL. In another example, the pharmaceutical composition is isotonic. In another example, the pharmaceutical composition contains: between 20 mM and 150 mM NaCl; between 5 mM and 100 mM arginine; and between 0.5% and 2% (w/v) sucrose. In another example, the pharmaceutical composition comprises a surfactant. In another example, the surfactant is polysorbate 20, polysorbate 80, or poloxamer 188. In another example, the surfactant is polysorbate 20 at a concentration (w/v) of between 0.001% and 0.1%. In another example, the surfactant is polysorbate 80 at a concentration (w/v) of between 0.001% and 0.1%. In another example, the surfactant is poloxamer 188 at a concentration (w/v) of between 0.01% to 0.3%. In another example, the pharmaceutical composition maintains a pH of between 5.8 and 6.7 for at least

two weeks when stored at approximately 25° C, and wherein less than 6% of the total etanercept is aggregated in a high molecular weight form as assessed using size exclusion chromatography. In another example, the pharmaceutical composition maintains a pH of between about 6.1 and about 6.5. In another example, less than 28% of the total amount of etanercept is in a misfolded form as assessed using hydrophobic interaction chromatography. In another example, the pharmaceutical composition consists essentially of about 40-100 mg/mL etanercept, about 120 mM NaCl, about 25 mM arginine, about 1% sucrose, and water. In another example, the pharmaceutical composition consists essentially of about 40-100 mg/mL etanercept, about 120 mM NaCl, about 25 mM arginine, about 1% sucrose, about 0.01% polysorbate 20, and water.

[0009] In another example, the present disclosure refers to a method of formulating a pharmaceutical composition of etanercept to remove an additional buffering agent and maintain pH from 6.1 to 6.5, comprising formulating the etanercept formulation in a formulation comprising an additional buffering agent at between pH 6.1 and 6.5, and exchanging the formulation comprising an additional buffering agent against a formulation that does not comprise an additional buffering agent and is between pH 5.6 and 6.5, and collecting the resulting pharmaceutical formulation. In one example, the exchange step uses diafiltration. In another example, the formulation that does not comprise an additional buffering agent is isotonic. In another example, the formulation that does not comprise an additional buffering agent contains sucrose, arginine, and NaCl. In another example, the formulation that does not comprise an additional buffering agent contains between 20 mM and 150 mM NaCl; between 5 mM and 100 mM arginine; and between 0.5% and 2% (w/v) sucrose. In another example, the formulation that does not comprise an additional buffering agent consists essentially of about 120 mM NaCl, about 25 mM arginine, about 1% sucrose, and water. In another example, the method of formulating a pharmaceutical composition of etanercept further comprises adding polysorbate. In another example, the polysorbate is polysorbate 20 at a concentration (w/v) of between 0.001% and 0.1%. In another example, the method of formulating a pharmaceutical composition of etanercept further comprises filtering the pharmaceutical composition. In another example, the method of formulating a pharmaceutical composition of etanercept further comprises aliquoting the pharmaceutical composition into a drug product form.

[0010] In another example, the present disclosure refers to a kit comprising a pharmaceutical

composition of etanercept as described above in a drug product form and instructions for storage and use.

[0011] In another example, the present disclosure refers to a pharmaceutical composition comprising etanercept, NaCl, arginine, sucrose, a phosphate buffer and benzyl alcohol, wherein the pH of the composition is between 6.1 and 6.5. In one example, the benzyl alcohol is at a concentration (v/v) of between 0.1% and 5.0%. In another example, the concentration of benzyl alcohol is about 0.9%. In another example, the pharmaceutical composition comprising etanercept further comprises polysorbate 20 at a concentration (w/v) of between 0.001% and 0.1%. In another example, the concentration of polysorbate 20 is about 0.004%. In another example, the pharmaceutical composition comprising etanercept consists essentially of etanercept at about 40-100 mg/mL, arginine at about 25 mM, sodium chloride at about 100 mM, sucrose at a concentration (w/v) of about 1%, phosphate buffer at about 25 mM, and benzyl alcohol at a concentration (v/v) of about 0.9%. In another example, the pharmaceutical composition comprising etanercept consists essentially of etanercept at about 40-100 mg/mL, arginine at about 25 mM, sodium chloride at about 100 mM, sucrose at a concentration (w/v) of about 1%, phosphate buffer at about 25 mM, benzyl alcohol at a concentration (v/v) of about 0.9%, and polysorbate 20 at a concentration (w/v) of about 0.004%.

[0012] In another example, the present disclosure refers to a single-dose container containing a pharmaceutical composition comprising etanercept as described above. In one example, the pharmaceutical composition consists essentially of etanercept at about 40-100 mg/mL, arginine at about 25 mM, sodium chloride at about 100 mM, sucrose at a concentration (w/v) of about 1%, phosphate buffer at about 25 mM, and benzyl alcohol at a concentration (v/v) of about 0.9%. In another example, the pharmaceutical composition consists essentially of etanercept at about 40-100 mg/mL, arginine at about 25 mM, sodium chloride at about 100 mM, sucrose at a concentration (w/v) of about 1%, phosphate buffer at about 25 mM, benzyl alcohol at a concentration (v/v) of about 0.9%, and polysorbate 20 at a concentration (w/v) of about 0.004%. In another example, the single dose container is a vial, a syringe, or an autoinjector. In another example, the single-dose container contains an aqueous formulation consisting of etanercept at 50.0 mg/mL, sodium chloride at 120 mM, L-arginine at 25 mM, sucrose at 1.0% (w/v).

[0013] In one aspect, the present disclosure refers to a method of preparing a single-dose

container, comprising filling the single-dose container with about a single dose of a pharmaceutical composition comprising between 75 mM and 150 mM NaCl, between 5 mM and 100 mM arginine, between 0.5% and 2% (w/v) sucrose, and between 40 mg/mL and 100 mg/mL etanercept, wherein the pharmaceutical composition comprises less than 2.0 mM total additional buffering agent, and the pH of the composition is between 6.1 and 6.5, under sterile conditions. In another aspect, there is described a method of preparing a single-dose container, comprising filling the single-dose container with about a single dose of the pharmaceutical composition comprising: about 50 mg/mL etanercept, wherein etanercept consists of a dimer of the amino acid sequence of SEQ ID NO: 1; about 120 mM NaCl; about 25 mM L-arginine hydrochloride; about 1 % (w/v) sucrose; and polysorbate 20 at a concentration (w/v0 of between about 0.001% and about 0.03%, under sterile conditions, wherein the pharmaceutical composition comprises less than 1.0 mM total additional buffering agent, wherein the pharmaceutical composition has an osmolality of about 290 to about 310 milliosmolals, and the pH of the composition is between 6.2 and 6.3. In a further aspect, there is described a method of preparing a pharmaceutical composition, the method comprising: purifying etanercept from a medium comprising cells expressing etanercept; and formulating etanercept to a formulation comprising: between 75 mM and 150 mM NaCl; between 5 mM and 100 mM arginine; between 0.5% and 2% (w/v) sucrose; and between 40 mg/mL and 100 mg/mL etanercept, wherein the pharmaceutical composition comprises less than 2.0 mM total additional buffering agent, and the pH of the composition is between 6.1 and 6.5.

Brief Description of the Drawings

[0014] Figure 1 shows the percent HMW (peak B) as detected by SEC for the etanercept stability assay of Example 3.

[0015] Figure 2 shows the percent LMW as detected by dSEC for the etanercept stability assay of Example 3.

[0016] Figure 3 shows the percent Peak 3 as detected by HIC for the etanercept stability assay of Example 3.

[0017] Figure 4 shows the percent Peak 3 as detected by HIC for the stainless steel cryo-vessel storage etanercept stability assay of Example 4.

[0018] Figure 5 shows the percent LMW as detected by dSEC for the stainless steel cryo-vessel storage etanercept stability assay of Example 4.

[0019] Figure 6 shows the percent Peak B as detected by SEC for the stainless steel cryo-

vessel storage etanercept stability assay of Example 4.

[0020] Figure 7 shows the percent Peak B as detected by SEC for the freeze-thaw etanercept stability assay of Example 4.

[0021] Figure 8 shows the pH stability of the conditioned AEX intermediate pool at controlled room temperature (CRT) in the assay of Example 6.

[0022] Figure 9 shows the UF/DF pool pH (A) and conductivity (B) stability at CRT in the assay of Example 6.

[0023] Figure 10 shows the pH (A) and conductivity (B) stability of etanercept formulated in SAS solution in the assay of Example 6.

Detailed Description of the Invention

[0024] The invention provides improved pharmaceutical compositions of etanercept. As used herein, the phrase "pharmaceutical composition" is understood to refer to a formulation of a polypeptide suitable for injection and/or administration into a patient in need thereof. More particularly, a pharmaceutical composition is substantially sterile and does not contain any agents that are unduly toxic or infectious to the recipient. Etanercept is a soluble form of the p75 TNF receptor fused to an Fc domain of a human IgG1 (TNFR:Fc). A commercially available

Claims

What is claimed is:

1. A method of preparing a single-dose container, comprising filling the single-dose container with about a single dose of a pharmaceutical composition comprising between 75 mM and 150 mM NaCl, between 5 mM and 100 mM arginine, between 0.5% and 2% (w/v) sucrose, and between 40 mg/mL and 100 mg/mL etanercept, wherein the pharmaceutical composition comprises less than 2.0 mM total additional buffering agent, and the pH of the composition is between 6.1 and 6.5, under sterile conditions.

2. The method of claim 1, wherein

(a) the pharmaceutical composition comprises less than 1.5 mM total additional buffering agent,

(b) the pharmaceutical composition comprises less than 0.5 mM total additional buffering agent,

(c) the arginine is L-arginine,

(d) etanercept consists of a dimer of the amino acid sequence of SEQ ID NO: 1, or

(e) the single-dose container is a syringe, a vial or an auto-injector.

3. The method of claim 1, wherein the pharmaceutical formulation comprises less than 0.25 mM total additional buffering agent.

4. The method of claim 3, wherein the arginine is L-arginine.5. The method of claim 4, wherein the pharmaceutical composition comprises about 50 mg/mL etanercept, about 120 mM NaCl, about 25 mM L-arginine hydrochloride, and about 1 % (w/v) sucrose.

6. The method of claim 5, wherein etanercept consists of a dimer of the amino acid sequence of SEQ ID NO: 1.

7. The method of claim 1, wherein the pharmaceutical composition comprises 0.1 mM or less total additional buffering agent.

8. The method of claim 7, wherein the pharmaceutical composition comprises about 50 mg/mL etanercept, about 120 mM NaCl, about 25 mM L-arginine hydrochloride, and about 1 % (w/v) sucrose.

9. The method of claim 1, wherein the pharmaceutical composition has an osmolality of about 180 to about 420 milliosmolals.

10. The method of claim 9, wherein the pharmaceutical composition has an osmolality of

about 300 to about 310 milliosmolals.

11. The method of claim 4, wherein the pharmaceutical composition consists essentially of about 50 mg/mL etanercept, about 120 mM NaCl, about 25 mM L-arginine hydrochloride, about 1% (w/v) sucrose, and water.

12. The method of claim 11, wherein etanercept consists of a dimer of the amino acid sequence of SEQ ID NO: 1.

13. The method of claim 1, wherein the pharmaceutical composition consists of about 50 mg/mL etanercept, about 120 mM NaCl, about 25 mM L-arginine hydrochloride, about 1% (w/v) sucrose, and water.

14. The method of claim 13, wherein etanercept consists of a dimer of the amino acid sequence of SEQ ID NO: 1.

15. The method of claim 1, wherein the pharmaceutical composition further comprises polysorbate 20.

16. The method of claim 15, wherein

(a) the polysorbate 20 concentration (w/v) is between about 0.001% and about 0.1%, or

(b) the polysorbate 20 concentration (w/v) is about 0.005%, about 0.01%, or about 0.015%.

17. A method of preparing a single-dose container, comprising filling the single-dose container with about a single dose of the pharmaceutical composition comprising:

about 50 mg/mL etanercept, wherein etanercept consists of a dimer of the amino acid sequence of SEQ ID NO: 1;

about 120 mM NaCl;

about 25 mM L-arginine hydrochloride;

about 1 % (w/v) sucrose; and

polysorbate 20 at a concentration (w/v) of between about 0.001% and about 0.03%,

under sterile conditions, wherein the pharmaceutical composition comprises less than 1.0 mM total additional buffering agent, wherein the pharmaceutical composition has an osmolality of about 290 to about 310 milliosmolals, and the pH of the composition is between 6.2 and 6.3.

18. The method of claim 17, wherein the pharmaceutical composition comprises polysorbate 20 at a concentration (w/v) of about 0.005%, about 0.01%, or about 0.015%.

19. A method of preparing a pharmaceutical composition, the method comprising:

purifying etanercept from a medium comprising cells expressing etanercept; and

formulating etanercept to a formulation comprising:

between 75 mM and 150 mM NaCl;

between 5 mM and 100 mM arginine;

between 0.5% and 2% (w/v) sucrose; and

between 40 mg/mL and 100 mg/mL etanercept,

wherein the pharmaceutical composition comprises less than 2.0 mM total additional buffering agent, and the pH of the composition is between 6.1 and 6.5.

20. The method of claim 19, wherein purifying comprises using one or more of chromatography, ethanol precipitation, gel electrophoresis and dialysis.

21. The method of claim 20, wherein purifying comprises using anion exchange chromatography.

22. The method of claim 21, wherein purifying comprises:

generating an intermediate pool from the medium via anion exchange chromatography, wherein the intermediate pool comprises between 40 mg/mL and 100 mg/mL etanercept; and

exchanging the intermediate pool with a formulation comprising between 75 mM and 150 mM NaCl, between 5 mM and 100 mM arginine, and between 0.5% and 2% (w/v) sucrose, wherein the formulation has a pH between 5.6 and 6.5, and does not comprise a buffering agent.

23. The method of claim 22, wherein the method further comprises conditioning the intermediate pool before exchanging, wherein the conditioned intermediate pool has a pH of between 6.2 and 6.4.

24. The method of claim 23, wherein exchanging comprises ultrafiltration.

25. The method of claim 19, wherein

(a) less than 28% of the total amount of etanercept in the pharmaceutical composition is in a misfolded form as assessed using hydrophobic interaction chromatography after two weeks' storage at approximately 25° C,

(b) said pharmaceutical composition maintains a pH of between about 6.1 and about 6.5 for at least two weeks during storage at approximately 25° C,

(c) the pharmaceutical composition maintains a pH between about 6.2 and about 6.3 when stored at controlled room temperature (CRT) for 2 weeks,

(d) the pharmaceutical composition maintains a pH of between 5.8 and 6.7 for at

least two weeks when stored at approximately 25° C, and wherein less than 6% of the total etanercept is aggregated in a high molecular weight form as assessed using size exclusion chromatography, or

(e) the method further comprises filling a single-dose container with about a single dose of the pharmaceutical composition.

26. The method of claim 19, further comprising adding a surfactant to the composition.

27. The method of claim 26, wherein the surfactant is polysorbate 20.