POLYSORBATE AND POLYOXYETHYLENE SORBITAN AS EXCIPIENTS FOR STABLE PROTEIN FORMULATIONS

RELATED APPLICATION

This application claims priority to U.S. Provisional Application Serial No. 63/565,861, filed on March 15, 2024, the entirety of which is incorporated herein by reference.

FIELD OF THE INVENTIONS

[0001] The present inventions provide hydrolyzed polysorbates and polyoxyethylene sorbitans (POES) alone or in combination with one or more polysorbate types in protein formulations in order to prevent surface absorption, degradation, and aggregation during agitation. The inventions also provide methods of making POESs and using POESs, alone or in combination with polysorbate types, in protein compositions, as well as in therapeutic and pharmaceutical formulations.

BACKGROUND OF THE INVENTIONS

[0002] Polysorbates, such as polysorbate 20 (PS20) and polysorbate 80 (PS80), are widely used surfactants in biopharmaceutical formulations to prevent proteins from denaturation, aggregation, agitation stress, surface adsorption and flocculant formation during activity inside and outside of the laboratory (i.e., processing, transporting, lyophilizing, and handling) (Yi, *et al.* 2020. "Polysorbate, the Good, the Bad and the Ugly." *American Pharmaceutical Review*, 26 Oct. 2020). Polysorbates are nonionic surfactants derived from ethoxylated sorbitan or isosorbide esterified with fatty acids (Yi, *et al.* 2020). Aside from those molecules esterified together, there are some other compounds within the heterogeneous solution. For example, the main fatty acid (FA) for polysorbate 80 (PS80) is oleic acid, however, the oleic acid content for PS80 at multi-compendial (MC) grade and Chinese pharmacopeia (ChP) grade is 58% and 98%, respectively (Liu, *et al.* 2022. Characterization of Polysorbate 80 by Liquid Chromatography-Mass Spectrometry to Understand Its Susceptibility to Degradation and Its Oxidative Degradation Pathway. *Journal*

of Pharmaceutical Sciences, 111(2), 323–334). In addition, the main FA for polysorbate 20 (PS20) is lauric acid, but that only takes up 40%-60% of all the esterified FA in MC-grade PS20 (Wuchner, *et al.* 2022. Industry Perspective on the Use and Characterization of Polysorbates for Biopharmaceutical Products Part 2: Survey Report on Control Strategy Preparing for the Future. *Journal of Pharmaceutical Sciences*, 111(11), 2955–2967.).

[0003] Although not being bound by any theory or hypothesis, it is believed that there are two main ways polysorbates (PS) prevent aggregation. First, they involve PS forming micelles around the protein, preventing the molecules from aggregating to each other or interacting with other surfaces. Second, PS settles on the air-liquid or surface-liquid interface, preventing proteins from contacting the air or surface which would cause the proteins to aggregate. Because of their protective traits as a nonionic surfactant, polysorbates are the top choice surfactant in the pharmaceutical industry.

[0004] Polysorbate degradation is a process in which the ester bond between the hydrophilic head and hydrophobic head is severed, creating polyoxyethylene sorbitan and free fatty acids (FFAs). Some proteins, such as mAb formulations are contaminated by lipases or esterases (host cell proteins) that are difficult to remove during purification. Lipases and esterases are often able to hydrolyze polysorbates. When polysorbates are hydrolyzed, FFAs (such as oleic acid and lauric acid) are released, which form particles in a drug product, rendering the end pharmaceutical product unsafe for use. The biopharmaceutical drug product is then deemed unusable because of protein aggregation or other particle formation caused by the FFAs released during PS hydrolysis.

SUMMARY OF THE INVENTIONS

[0005] The inventions provide methods for producing stabilized protein formulations, wherein any method thereof can comprise the steps of: (a) providing a protein solution, wherein the concentration of protein is about 5 to 300 mg/ml; and (b) adding hydrolyzed polysorbate (hPS), polyoxyethylene sorbitan (POES, such as isolated POES), and/or synthetic POES (sPOES), optionally in combination with one or more polysorbate types to the solution to a concentration of about 0.01 to 0.30% (w/v) to provide a stabilized formulation. The stabilized formulations can exhibit less than a 0.5% increase in aggregation after agitation as measured by size exclusion ultra performance liquid chromatography (SE-UPLC); and/or less

than a 0.05 AU increase insoluble aggregates after agitation as measured by a spectrophotometer at OD405 nm. For example, the agitation can be carried out by shaking the protein formulations in an upright orientation for about 60 to 120 minutes at about 1000 rpm, for example, and in a sideways orientation for about 24 to 48 hours at about 250 rpm, for example, at room temperature. The concentration of protein in the stabilized formulations can be about 10 to 200 mg/ml, about 50 to 150 mg/ml, about 50 mg/ml, or about 150 mg/ml, for example. The hPS in the stabilized formulations can be hydrolyzed polysorbate 80 (hPS80) or hydrolyzed polysorbate 20 (hPS20). The POES in the stabilized formulations can be isolated POES or sPOES. The concentration of the hPS and/or POESs, optionally in combination with one or more polysorbate types in the stabilized formulations can be about 0.001 to 0.25% (w/v), about 0.05 to 0.10% (w/v), about 0.10 to 0.15% (w/v), about 0.15 to 0.20% (w/v), about 0.20 to 0.25% (w/v), or about 0.25 to 0.30% (w/v).

[0006] The concentration of the hPS, isolated POES, and/or sPOES, optionally in combination with one or more polysorbate types in the stabilized formulations also can be about 0.001 % (w/v), about 0.02 % (w/v), about 0.03 % (w/v), about 0.04 % (w/v), about 0.05 % (w/v), about 0.06 % (w/v), about 0.07 % (w/v), about 0.08 % (w/v), about 0.09 % (w/v), about 0.1 % (w/v), about 0.11 % (w/v), about 0.12 % (w/v), about 0.13 % (w/v), about 0.14 % (w/v), about 0.15 % (w/v), about 0.16 % (w/v), about 0.17 % (w/v), about 0.18 % (w/v), about 0.19 % (w/v), about 0.2 % (w/v), about 0.21 % (w/v), about 0.22 % (w/v), about 0.23 % (w/v), about 0.24 % (w/v), about 0.25 % (w/v), about 0.26 % (w/v), about 0.27 % (w/v), about 0.28 % (w/v), about 0.29 % (w/v), about 0.30 % (w/v). The protein in the stabilized formulations can be antibodies, antibody fragments or antibody derivatives, wherein the protein can be an Fc-containing protein. The Fc-containing protein can be a receptor-Fc-fusion protein, wherein the receptor-Fc-fusion protein can be a Trap protein. The protein in the stabilized formulations can be an antibody, a monoclonal antibody or an IgG antibody. The stabilized formulations can be a liquid formulation, wherein the liquid formulations can comprise pharmaceutically acceptable excipients including one or more buffer, salt and sugar.

[0007] The inventions also provide stabilized protein formulations produced by methods, wherein any method thereof can comprise the steps of: (a) providing a protein solution, wherein the concentration of protein is about 5 to 300 mg/ml; and (b) adding hydrolyzed polysorbate (hPS or hydrolyzed PS), polyoxyethylene sorbitan (POES), and/or

sPOES, optionally in combination with one or more polysorbate types to the solution to a concentration of about 0.01 to 0.30% (w/v) to provide a stabilized formulation. The stabilized protein formulations of the inventions are resistant to agitation. The stabilized formulations can exhibit less than a 0.5% increase in aggregation after agitation as measured by SE-UPLC; and/or less than a 0.05 AU increase insoluble aggregates after agitation as measured by a spectrophotometer at OD405 nm. For example, the agitation can be carried out by shaking the protein formulations in an upright orientation for about 60 to 120 minutes at about 1000 rpm, for example, and in a sideways orientation for about 24 to 48 hours at about 250 rpm, for example, at room temperature. The concentration of protein in the stabilized formulations can be about 10 to 200 mg/ml, about 50 to 150 mg/ml, about 50 mg/ml, or about 150 mg/ml, for example. The hPS in the stabilized formulations can be hydrolyzed polysorbate 80 (hPS80) or hydrolyzed polysorbate 20 (hPS20). The POES in the stabilized formulations can be isolated POES or sPOES.

[0008] The concentration of the hPS, sPOES and/or the POES in the stabilized formulations can be about 0.001 to 0.25% (w/v), about 0.05 to 0.10% (w/v), about 0.10 to 0.15% (w/v), about 0.15 to 0.20% (w/v), about 0.20 to 0.25% (w/v), or about 0.25 to 0.30% (w/v). The concentration of the hPS, the sPOES and/or the POES in the stabilized formulations also can be about 0.01 % (w/v), about 0.02 % (w/v), about 0.03 % (w/v), about 0.04 % (w/v), about 0.05 % (w/v), about 0.06 % (w/v), about 0.07 % (w/v), about 0.08 % (w/v), about 0.09 % (w/v), about 0.1 % (w/v), about 0.11 % (w/v), about 0.12 % (w/v), about 0.13 % (w/v), about 0.14 % (w/v), about 0.15 % (w/v), about 0.16 % (w/v), about 0.17 % (w/v), about 0.18 % (w/v), about 0.19 % (w/v), about 0.2 % (w/v), about 0.21 % (w/v), about 0.22 % (w/v), about 0.23 % (w/v), about 0.24 % (w/v), about 0.25 % (w/v), about 0.26 % (w/v), about 0.27 % (w/v), about 0.28 % (w/v), about 0.29 % (w/v), about 0.30 % (w/v). The protein in the stabilized formulations can be antibodies, antibody fragments or antibody derivatives, wherein the protein can be an Fc-containing protein. The Fc-containing protein can be a receptor-Fc-fusion protein, wherein the receptor-Fc-fusion protein can be a Trap protein. The protein in the stabilized formulations can be an antibody, a monoclonal antibody or an IgG antibody. The stabilized formulations can be a liquid formulation, wherein the liquid formulations can comprise pharmaceutically acceptable excipients including one or more buffer, salt and sugar.

[0009] The inventions provide methods for producing stabilized protein formulations, wherein any method thereof can comprise the steps of: (a) providing a protein solution, wherein the concentration of protein is about 5 to 300 mg/ml; and (b) adding hydrolyzed polysorbate (hPS), sPOES, and/or polyoxyethylene sorbitan (POES) at a concentration of about 0.01 to 0.30% (w/v) and about 0.01% (w/v) to about 0.30% (w/v) of PS80 or PS20 to the solution to provide a stabilized formulation. The stabilized formulations can exhibit less than a 0.5% increase in aggregation after agitation as measured by size exclusion ultra performance liquid chromatography (SE-UPLC); and/or less than a 0.05 AU increase insoluble aggregates after agitation as measured by a spectrophotometer at OD405 nm. For example, the agitation can be carried out by shaking the protein formulations in an upright orientation for about 60 to 120 minutes at about 1000 rpm, for example, and in a sideways orientation for about 24 to 48 hours at about 250 rpm, for example, at room temperature. The concentration of protein in the stabilized formulations can be about 10 to 200 mg/ml, about 50 to 150 mg/ml, about 50 mg/ml, or about 150 mg/ml. The hPS in the stabilized formulations can be hydrolyzed polysorbate 80 (hPS80) or hydrolyzed polysorbate 20 (hPS20). The POES in the stabilized formulations can be POES (such as isolated POES) and/or sPOES. The concentration of the hPS, the sPOES, and/or the POES, optionally in combination with one or more polysorbate types in the stabilized formulations can be about 0.001 to 0.25% (w/v), about 0.05 to 0.10% (w/v), about 0.10 to 0.15% (w/v), about 0.15 to 0.20% (w/v), about 0.20 to 0.25% (w/v), or about 0.25 to 0.30% (w/v). The concentration of the hPS, the sPOES, and/or the POES in the stabilized formulations also can be about 0.01 % (w/v), about 0.02 % (w/v), about 0.03 % (w/v), about 0.04 % (w/v), about 0.05 % (w/v), about 0.06 % (w/v), about 0.07 % (w/v), about 0.08 % (w/v), about 0.09 % (w/v), about 0.1 % (w/v), about 0.11 % (w/v), about 0.12 % (w/v), about 0.13 % (w/v), about 0.14 % (w/v), about 0.15 % (w/v), about 0.16 % (w/v), about 0.17 % (w/v), about 0.18 % (w/v), about 0.19 % (w/v), about 0.2 % (w/v), about 0.21 % (w/v), about 0.22 % (w/v), about 0.23 % (w/v), about 0.24 % (w/v), about 0.25 % (w/v), about 0.26 % (w/v), about 0.27 % (w/v), about 0.28 % (w/v), about 0.29 % (w/v), about 0.30 % (w/v). The protein in the stabilized formulations can be antibodies, antibody fragments or antibody derivatives, wherein the protein can be an Fc-containing protein. The Fc-containing protein can be a receptor-Fc-fusion protein, wherein the receptor-Fc-fusion protein can be a Trap protein. The protein in the stabilized formulations can be an antibody, a monoclonal antibody or an IgG antibody. The stabilized formulations can be a liquid formulation, wherein the liquid

formulations can comprise pharmaceutically acceptable excipients including one or more buffer, salt and sugar.

[0010] The inventions also provide stabilized protein formulations produced by methods, wherein any method thereof can comprise the steps of: (a) providing a protein solution, wherein the concentration of protein is about 5 to 300 mg/ml; and (b) adding hydrolyzed polysorbate (hPS), synthetic polyoxyethylene sorbitan (sPOES), and/or polyoxyethylene sorbitan (POES, such as isolated POES), optionally in combination with one or more polysorbate types at a concentration of about 0.01 to 0.30% (w/v) and about 0.001% (w/v) to about 0.30% (w/v) of PS80 or PS20 to the solution to provide a stabilized formulation. The stabilized protein formulations of the inventions are resistant to agitation. The stabilized formulations can exhibit less than a 0.5% increase in aggregation after agitation as measured by SE-UPLC; and/or less than a 0.05 AU increase insoluble aggregates after agitation as measured by a spectrophotometer at OD405 nm. For example, the agitation can be carried out by shaking the protein formulations in an upright orientation for about 60 to 120 minutes at about 1000 rpm, for example, and in a sideways orientation for about 24 to 48 hours at about 250 rpm, for example, at room temperature. The concentration of protein in the stabilized formulations can be about 10 to 200 mg/ml, about 50 to 150 mg/ml, about 50 mg/ml, or about 150 mg/ml. The hPS in the stabilized formulations can be hydrolyzed polysorbate 80 (hPS80) or hydrolyzed polysorbate 20 (hPS20). The POES in the stabilized formulations can be isolated POES or sPOES. The concentration of the hPS, the sPOES, and/or the POES, optionally in combination with one or more polysorbate types in the stabilized formulations can be about 0.001 to 0.25% (w/v), about 0.05 to 0.10% (w/v), about 0.10 to 0.15% (w/v), about 0.15 to 0.20% (w/v), about 0.20 to 0.25% (w/v), or about 0.25 to 0.30% (w/v). The concentration of the hPS, the sPOES, or the POES in the stabilized formulations also can be about 0.01 % (w/v), about 0.02 % (w/v), about 0.03 % (w/v), about 0.04 % (w/v), about 0.05 % (w/v), about 0.06 % (w/v), about 0.07 % (w/v), about 0.08 % (w/v), about 0.09 % (w/v), about 0.1 % (w/v), about 0.11 % (w/v), about 0.12 % (w/v), about 0.13 % (w/v), about 0.14 % (w/v), about 0.15 % (w/v), about 0.16 % (w/v), about 0.17 % (w/v), about 0.18 % (w/v), about 0.19 % (w/v), about 0.2 % (w/v), about 0.21 % (w/v), about 0.22 % (w/v), about 0.23 % (w/v), about 0.24 % (w/v), about 0.25 % (w/v), about 0.26 % (w/v), about 0.27 % (w/v), about 0.28 % (w/v), about 0.29 % (w/v), about 0.30 % (w/v). The protein in the stabilized formulations can be antibodies, antibody fragments or antibody derivatives, wherein the protein can be an Fc-containing

protein. The Fc-containing protein can be a receptor-Fc-fusion protein, wherein the receptor-Fc-fusion protein can be a Trap protein. The protein in the stabilized formulations can be an antibody, a monoclonal antibody or an IgG antibody. The stabilized formulations can be a liquid formulation, wherein the liquid formulations can comprise pharmaceutically acceptable excipients including one or more buffer, salt and sugar.

BRIEF DESCRIPTION OF THE FIGURES

[0011] **Figure 1A-1D** depict molecular structures of excipients polysorbate 80 (PS80), polysorbate 20 (PS20), Poloxamer 188, and polyethylene glycol-3350 (PEG-3350), respectively. **Figure 1E** illustrates hydrophobic tail and hydrophilic head of a surfactant (Polysorbate).

[0012] **Figure 2** schematically illustrates hydrolysis of polysorbates and purification of POES. **Figure 2 also** demonstrates the translation of the nominal value of POES percentage (%) to the actual percentage (%).

[0013] **Figure 3** depicting hydrolyzed structure of polysorbate 80 (PS80), polyoxyethylene sorbitan (POES).

[0014] **Figure 4A and 4B** showing chemical degradation of PS80 and PS20, respectively. Total ion chromatogram (TIC) graphs of both regular polysorbate 80 and 20 are compared with the TIC graph of polyoxyethylene sorbitan (POES). The PS80 and PS20 lines illustrate normal polysorbate behavior, while the POES lines illustrate degraded POES behavior. The smaller peaks at the beginning of each graph represent the POE, POE sorbitan, and the POE isosorbide that is present in both polysorbates and the POES solution. Although, the POE sorbitan concentration exceeds the concentrations of the other two components. The highest peaks in the PS20 and PS80 graphs are various intact polysorbates. While the POES lines show many of these peaks, the POES line stay almost completely flat, indicating that there was little to no intact polysorbates left in the POES solution.

[0015] **Figure 5** illustrates the change in high molecular weight percentage (HMW%) vs. the excipient concentrations for hydrolyzed polysorbate 80 compared to regular PS80 using mAb1, which is dupilumab at a concentration of 150 mg/ml. The graph shows the trend for non-hydrolyzed PS80 is regular: as the concentration of PS80 increases, the amount of damaged protein decreases.

[0016] **Figure 6** is a graph showing the relationship between polysorbates 80 (non-hydrolyzed PS80 and isolated POES) and the change in HMW% in the samples using mAb1 (dupilumab) at a concentration of 150 mg/ml.

[0017] **Figure 7** is a graph showing the relationship between polysorbates 80 (non-hydrolyzed PS80 and isolated POES) and the change in ΔOD of the samples using mAb1 (dupilumab) at a concentration of 150 mg/ml.

[0018] **Figure 8** depicts charged aerosol detector (CAD) data, graph showing the protective effects of the hydrolyzed polysorbates are not caused by the polysorbate residue.

[0019] **Figure 9** is a graph demonstrating the relationship between the concentration of sodium oleate in place of polysorbate and the change in HMW concentration.

[0020] **Figure 10** is a graph showing the relationship between polysorbates 20 (non-hydrolyzed PS20 and isolated POES) and the change in ΔOD of the samples using mAb1 (dupilumab) at a concentration of 150 mg/ml.

[0021] **Figure 11** is a graph showing the relationship between hydrolyzed PS20 and change in HMW concentration compared to regular PS20 in the samples using mAb1 (dupilumab) at a concentration of 150 mg/ml.

[0022] **Figure 12** is a graph showing the relationship between the concentration of POES to the change in concentration of HMW compared to regular PS20 in the samples using mAb1 (dupilumab) at a concentration of 150 mg/ml.

[0023] **Figure 13** is a graph showing the relationship between non-hydrolyzed PS80 and isolated POES and the change in HMW% in the samples using mAb1 (dupilumab) at a concentration of 150 mg/ml in an experiment that involved shaking protein solutions for 48 hours at 25°C.

[0024] **Figure 14** is a graph showing the relationship between non-hydrolyzed PS80 and isolated POES and the change in ΔOD of the samples using mAb1 (dupilumab) at a concentration of 150 mg/ml in an experiment that involved shaking protein solutions for 48 hours at 25°C.

[0025] **Figure 15** is a graph showing the relationship between non-hydrolyzed PS80 and isolated POES and the change in HMW% in the samples using mAb2 (an antibody targeting Bet v 1) at a concentration of 150 mg/ml.

[0026] **Figure 16** is a graph showing the relationship between non-hydrolyzed PS80 and isolated POES and the change in ΔOD of the samples using mAb2 (targeting Bet v 1) at a concentration of 150 mg/ml.

[0027] **Figure 17** is a graph showing that POES protects mAb2 (targeting Bet v 1) at a concentration of 50 mg/ml from agitation stress.

[0028] **Figure 18** is a graph showing the relationship between non-hydrolyzed PS80 and isolated POES and the change in HMW% in the samples using mAb3 (another antibody targeting Bet v 1) at a concentration of 150 mg/ml.

[0029] **Figure 19** is a graph showing the relationship between non-hydrolyzed PS80 and isolated POES and the change in ΔOD of the samples using mAb3 (targeting Bet v 1) at a concentration of 150 mg/ml.

[0030] **Figure 20** is a graph showing the relationship between non-hydrolyzed PS20 and isolated POES and the change in HMW% in the samples using mAb3 (targeting Bet v 1) at a concentration of 150 mg/ml.

[0031] **Figure 21** illustrates the effect of agitation stress and the change in HMW% in the samples as labeled for polysorbate type, temperature and time.

[0032] **Figures 22A to 22C** illustrate thermal stability of mAb1 (dupilumab) in the presence of POES or PS80 at 5° C (**Fig. 22A**), 25° C (**Fig. 22B**) and 40° C (**Fig. 22C**).

[0033] **Figures 23A to 23C** illustrate surfactant degradation in lipase-containing mAb1 (dupilumab) formulations during thermal stability with POES or PS80 at 5° C (**Fig. 23A**), 25° C (**Fig. 23B**) and 40° C (**Fig. 23C**).

[0034] **Figure 24** is a graph showing subvisible particle (SVP) counts in lipase-containing mAb (dupilumab)formulations during thermal stability at 40° C.

[0035] **Figures 25A to 25D** illustrate protection of mAb1 (dupilumab) from agitation by combining POES and PS80. **Figures 25A** (POES alone), **25B** (PS80 alone), **25C** (POES combined with 0.005% PS80), and **25D** (POES combined with 0.01% PS80) illustrate the change in high molecular weight percentage (HMW%) versus the surfactant concentrations alone or in combinations.

[0036] **Figure 26** is a graph showing the effects of combination of POES and PS80 in protecting mAb (dupilumab) formulations from agitation stress.

[0037] **Figures 27A and 27B** illustrate protection of mAb (dupilumab) formulations from agitation stress by combining POES and PS80. **Figures 27A** (PS80 alone) and **27B** (POES combined with no PS80, 0.005% PS80, or 0.01% PS80) illustrate the change in turbidity (ΔOD405) versus the surfactant concentrations.

[0038] **Figure 28** schematically illustrates the effect of synthetic POES (sPOES) on protein surface binding by Quartz Crystal Microbalance with Dissipation (QCM-D) monitoring.

[0039] **Figure 29** is a bar graph showing the effect of combining POES with PS80 on the absorption of mAb1 (dupilumab) to Polyvinylidene difluoride (PVDF) membrane and Protein-PVDF interaction (usually used as filter material).

DETAILED DESCRIPTION OF THE INVENTIONS

DEFINITIONS

[0040] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0041] The term “about” in the context of numerical values and ranges refers to values or ranges that approximate or are close to the recited values or ranges such that the invention can perform as intended, such as having a desired rate, amount, density, degree, increase, decrease, percentage, value, purity, pH, concentration, presence of a form or variant, temperature or amount of time, as is apparent from the teachings contained herein. For example, “about” can signify values either above or below the stated value in a range of approx. +/- 10% or more or less depending on the ability to perform. Thus, this term encompasses values beyond those simply resulting from systematic error.

[0042] “Antibodies” (also referred to as "immunoglobulins") are examples of proteins having multiple polypeptide chains and extensive post-translational modifications. The canonical immunoglobulin protein (for example, IgG) comprises four polypeptide chains - two light chains and two heavy chains. Each light chain is linked to one heavy chain via a cysteine disulfide bond, and the two heavy chains are bound to each other via two cysteine disulfide bonds. Immunoglobulins produced in mammalian systems are also glycosylated at various residues (for example, at asparagine residues) with various polysaccharides, and can

differ from species to species, which may affect antigenicity for therapeutic antibodies. Butler and Spearman, "The choice of mammalian cell host and possibilities for glycosylation engineering", *Curr. Opin. Biotech.* 30:107-112 (2014).

[0043] Antibodies are often used as therapeutic biomolecules. An antibody includes immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region comprises three domains, CH1, CH2 and CH3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region comprises one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 (heavy chain CDRs may be abbreviated as HCDR1, HCDR2 and HCDR3; light chain CDRs may be abbreviated as LCDRl, LCDR2 and LCDR3. The term "high affinity" antibody refers to those antibodies having a binding affinity to their target of at least 10-9 M, at least 10-10 M; at least 10-11 M; or at least 10-12 M, as measured by surface plasmon resonance, for example, BIACORE™ or solution-affinity ELISA.

[0044] The phrase "bispecific antibody" includes an antibody capable of selectively binding two or more epitopes. Bispecific antibodies generally comprise two different heavy chains, with each heavy chain specifically binding a different epitope -- either on two different molecules (for example, antigens) or on the same molecule (for example, on the same antigen). If a bispecific antibody is capable of selectively binding two different epitopes (a first epitope and a second epitope), the affinity of the first heavy chain for the first epitope will generally be at least one to two, three or four orders of magnitude lower than the affinity of the first heavy chain for the second epitope, and vice versa. The epitopes recognized by the bispecific antibody can be on the same or a different target (for example, on the same or a different protein). Bispecific antibodies can be made, for example, by combining heavy chains that recognize different epitopes of the same antigen. For example, nucleic acid sequences encoding heavy chain variable sequences that recognize different

epitopes of the same antigen can be fused to nucleic acid sequences encoding different heavy chain constant regions, and such sequences can be expressed in a cell that expresses an immunoglobulin light chain. A typical bispecific antibody has two heavy chains each having three heavy chain CDRs, followed by (N-terminal to C-terminal) a CH1 domain, a hinge, a CH2 domain, and a CH3 domain, and an immunoglobulin light chain that either does not confer antigen-binding specificity but that can associate with each heavy chain, or that can associate with each heavy chain and that can bind one or more of the epitopes bound by the heavy chain antigen-binding regions, or that can associate with each heavy chain and enable binding or one or both of the heavy chains to one or both epitopes.

[0045] The phrase "heavy chain," or "immunoglobulin heavy chain" includes an immunoglobulin heavy chain constant region sequence from any organism, and unless otherwise specified includes a heavy chain variable domain. Heavy chain variable domains include three heavy chain CDRs and four FR regions, unless otherwise specified. Fragments of heavy chains include CDRs, CDRs and FRs, and combinations thereof. A typical heavy chain has, following the variable domain (from N-terminal to C-terminal), a CH1 domain, a hinge, a CH2 domain, and a CH3 domain. A functional fragment of a heavy chain includes a fragment that is capable of specifically recognizing an antigen (for example, recognizing the antigen with a KD in the micromolar, nanomolar, or picomolar range), that is capable of expressing and secreting from a cell, and that comprises at least one CDR.

[0046] The phrase "light chain" includes an immunoglobulin light chain constant region sequence from any organism, and unless otherwise specified includes human kappa and lambda light chains. Light chain variable (VL) domains typically include three light chain CDRs and four framework (FR) regions, unless otherwise specified. Generally, a full-length light chain includes, from amino terminus to carboxyl terminus, a VL domain that includes FR1-CDR1- FR2-CDR2-FR3-CDR3-FR4, and a light chain constant domain. Light chains that can be used with these inventions include those, for example, that do not selectively bind either the first or second antigen selectively bound by the antigen-binding protein. Suitable light chains include those that can be identified by screening for the most commonly employed light chains in existing antibody libraries (wet libraries or in silico), where the light chains do not substantially interfere with the affinity and/or selectivity of the antigen-binding domains of the antigen-binding proteins. Suitable light chains include those

that can bind one or both epitopes that are bound by the antigen-binding regions of the antigen-binding protein.

[0047] The phrase "variable domain" includes an amino acid sequence of an immunoglobulin light or heavy chain (modified as desired) that comprises the following amino acid regions, in sequence from N-terminal to C-terminal (unless otherwise indicated): FRl, CDRl, FR2, CDR2, FR3, CDR3, FR4. A "variable domain" includes an amino acid sequence capable of folding into a canonical domain (VH or VL) having a dual beta sheet structure wherein the beta sheets are connected by a disulfide bond between a residue of a first beta sheet and a second beta sheet.

[0048] The phrase "complementarity determining region" ("CDR") includes an amino acid sequence encoded by a nucleic acid sequence of an organism's immunoglobulin genes that normally (that is, in a wild-type organism) appears between two framework regions in a variable region of a light or a heavy chain of an immunoglobulin molecule (for example, an antibody or a T cell receptor). A CDR can be encoded by, for example, a germline sequence or a rearranged or unrearranged sequence, and, for example, by a naive or a mature B cell or a T cell. In some circumstances (for example, for a CDR3), CDRs can be encoded by two or more sequences (for example, germline sequences) that are not contiguous (for example, in a nucleic acid sequence that has not been rearranged) but are contiguous in a B cell nucleic acid sequence, for example, as the result of splicing or connecting the sequences (for example, V-D-J recombination to form a heavy chain CDR3).

[0049] “Antibody derivatives and fragments” include, but are not limited to: antibody fragments (for example, ScFv-Fc, dAB-Fc, half antibodies, Fab), multispecifics (for example, IgG-ScFv, IgG-dab, ScFV-Fc-ScFV, tri-specific). “Fab” refers to an antibody fragment comprising an antigen binding region. An Fab typically will lack the Fc portion.

[0050] The phrase "Fc-containing protein" includes antibodies, bispecific antibodies, antibody derivatives containing an Fc, antibody fragments containing an Fc, Fc-fusion proteins, receptor Fc-fusion proteins (including trap proteins), immunoadhesins, and other binding proteins that comprise at least a functional portion of an immunoglobulin CH2 and CH3 region. A "functional portion" refers to a CH2 and CH3 region that can bind a Fc receptor (for example, an FcyR; or an FcRn, (neonatal Fc receptor), and/or that can participate in the activation of complement. If the CH2 and CH3 region contains deletions, substitutions, and/or insertions or other modifications that render it unable to bind any Fc

receptor and also unable to activate complement, the CH2 and CH3 region is not functional. Fc-fusion proteins include, for example, Fc-fusion (N-terminal), Fc-fusion (C-terminal), mono-Fc-fusion and bispecific Fc-fusion proteins.

[0051] "Fc" stands for fragment crystallizable, and is often referred to as a fragment constant. Antibodies contain an Fc region that is made up of two identical protein sequences. IgG has heavy chains known as γ-chains. IgA has heavy chains known as α-chains, IgM has heavy chains known as μ-chains. IgD has heavy chains known as σ-chains. IgE has heavy chains known as ε-chains. In nature, Fc regions are the same in all antibodies of a given class and subclass in the same species. Human IgGs have four subclasses and share about 95% homology amongst the subclasses. In each subclass, the Fc sequences are the same. For example, human IgG1 antibodies will have the same Fc sequences. Likewise, IgG2 antibodies will have the same Fc sequences; IgG3 antibodies will have the same Fc sequences; and IgG4 antibodies will have the same Fc sequences. Alterations in the Fc region create charge variation.

[0052] Fc-containing proteins, such as antibodies, can comprise modifications in immunoglobulin domains, including where the modifications affect one or more effector function of the binding protein (for example, modifications that affect FcyR binding, FcRn binding and thus half-life, and/or CDC activity). Such modifications include, but are not limited to, the following modifications and combinations thereof, with reference to EU numbering of an immunoglobulin constant region: 238, 239, 248, 249, 250, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 297, 298, 301, 303, 305, 307, 308, 309, 311, 312, 315, 318, 320, 322, 324, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 337, 338, 339, 340, 342, 344, 356, 358, 359, 360, 361, 362, 373, 375, 376, 378, 380, 382, 383, 384, 386, 388, 389, 398, 414, 416, 419, 428, 430, 433, 434, 435, 437, 438, and 439.

[0053] For example, and not by way of limitation, the binding protein is an Fc-containing protein (for example, an antibody) and exhibits enhanced serum half-life (as compared with the same Fc-containing protein without the recited modification(s)) and have a modification at position 250 (for example, E or Q); 250 and 428 (for example, L or F); 252 (for example, L/Y/F/W or T), 254 (for example, S or T), and 256 (for example, S/R/Q/E/D or T); or a modification at 428 and/or 433 (for example, L/R/SI/P/Q or K) and/or 434 (for example, H/F or Y); or a modification at 250 and/or 428; or a modification at 307 or 308 (for

example, 308F, V308F), and 434. In another example, the modification can comprise a 428L (for example, M428L) and 434S (for example, N434S) modification; a 428L, 2591 (for example, V259I), and a 308F (for example, V308F) modification; a 433K (for example, H433K) and a 434 (for example, 434Y) modification; a 252, 254, and 256 (for example, 252Y, 254T, and 256E) modification; a 250Q and 428L modification (for example, T250Q and M428L); a 307 and/or 308 modification (for example, 308F or 308P).

[0054] "Fv" stands for fragment variable, and is primarily responsible for binding to epitopes.

[0055] As used herein, the expression "formulation" means a combination of at least one active ingredient (e.g., a protein, such as polypeptide, antibody, monoclonal antibody, etc. which is capable of exerting a biological effect in a human or non-human animal), and at least one inactive ingredient which, when combined with the active ingredient or one or more additional inactive ingredients, is suitable for therapeutic administration to a human or non-human animal. The term "formulation", as used herein, means "pharmaceutical" or "therapeutic" formulations unless specifically indicated otherwise. The present invention provides pharmaceutical formulations comprising at least one therapeutic protein. According to the present invention, the therapeutic protein is an antibody, or an antigen-binding fragment thereof. The term "formulation", as used herein, also means a solid, semisolid, and/or liquid formulation, such as suitable for oral, subcutaneous and/or intravenous administration.

[0056] "Stable protein formulation", as used herein, refers generally to protein formulations that are stable under tested agitation conditions. For example, general guidelines for testing agitation stability of drug products include shaking the glass vial containing the protein of interest (such as a protein drug, an antibody, antibody fragment, antibody derivative, an Fc-fusion protein, for example) in upright vial orientation for 60 and 120 minutes on vortexer at 500 to 1500 rpm (preferably about 1000 rpm) at room temperature and in sideways vial orientation for 24 and 48 hours on orbital shaker at 100-400 rpm (preferably about 250 rpm) at room temperature. Formulations can be considered stable following agitation if at least one of (i) clarity is maintained (measured by visual inspection), (ii) aggregation (measured by Size-Exclusion-Ultra Performance Liquid Chromatography (SE-UPLC) exhibits less than a 0.5% increase in aggregation) or (iii) insoluble aggregates or turbidity increase (measured by OD405 nm exhibits less than a 0.05 AU increase).

[0057] Various methods of stability testing are known in the art, including: Fleischman et al. Shipping-Induced Aggregation in Therapeutic Antibodies: Utilization of a Scale-Down Model to Assess Degradation in Monoclonal Antibodies. J. Pharm. Sci. (2017) 106: 994-1000; Ghazvini et al. Evaluating the Role of the Air-Solution Interface on the Mechanism of Subvisible Particle Formation Caused by Mechanical Agitation for an IgG1 mAbI. J. Pharm. Sci. (2016) 105: 1643-1656; and Torisu et al. Synergistic Effect of Cavitation and Agitation on Protein Aggregation. J. Pharm. Sci. (2017) 106: 521-529).

[0058] "Polypeptide" or "peptide" refers to sequence(s) of amino acids covalently joined. Polypeptides include natural, semi-synthetic and synthetic proteins and protein fragments. "Polypeptide" and "protein" can be used interchangeably. Oligopeptides are considered shorter polypeptides.

[0059] "Protein of interest" or "polypeptide of interest" (POI) can have any amino acid sequence, and includes any protein, polypeptide, or peptide that is desired to be expressed, typically for gene therapy purposes. Protein types can include, but are not limited to, receptors, fusion proteins, agonists, antagonists, activators, inhibitors, enzymes (such as those used in enzyme replacement therapy), factors and co-factors, repressors, activators, ligands, protein hormones, structural proteins, storage proteins, transport proteins, signal proteins, neurotransmitters and contractile proteins. Derivatives, components, domains, chains and fragments of the above also are included. The sequences can be natural, semi-synthetic or synthetic.

[0060] All numerical limits and ranges set forth herein include all numbers or values thereabout or there between of the numbers of the range or limit. The ranges and limits described herein expressly denominate and set forth all integers, decimals and fractional values defined and encompassed by the range or limit. Thus, a recitation of ranges of values herein are merely intended to serve as a way of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein.

DESCRIPTION

Degradation of polysorbate and challenges in protecting proteins from aggregation

[0061] Polysorbate 80 (PS80), polysorbate 20 (PS20), Poloxamer 188, and polyethylene glycol-3350 (PEG-3350) can be added to protein formulations to prevent degradation during agitation caused by processing, transporting, lyophilizing, or handling. Generally, lipases in protein drug product degrade polysorbate and if the polysorbate degraded, the drug product is no longer considered stable.

[0062] Root causes of polysorbate degradation include enzymatic, oxidative, and chemical degradation, resulting in flocculant or particle formation, whether it be from free fatty acids (FFAs) or protein aggregates. Other causes of particle formation linked to polysorbate degradation include buffer excipients, salts, pH, protein and even the storage container. All of which compromise the integrity of the protein drug product (Dwivedi, et al. 2018. Polysorbate degradation in biotherapeutic formulations: Identification and discussion of current root causes. International journal of pharmaceutics, 552(1-2), 422–436).

[0063] The most pressing cause of polysorbate degradation is enzymatic hydrolysis. Enzymatic hydrolysis typically involves cleaving the polysorbate at the ester bond, leaving behind the polyoxyethylene sorbitan (POES) and the FFAs. Enzymatic polysorbate degradation is caused by a select few types of proteins, most notably, host-cell proteins. Host-cell proteins (HCPs) are considered as those problematic HCPs that are immunogenic, biologically active, or enzymatically active with the potential to degrade either product molecules or excipients (such as PS) used in formulations (Yi, et al. 2020. “Polysorbate, the Good, the Bad and the Ugly.” American Pharmaceutical Review, American Pharmaceutical Review, 26 Oct. 2020).

[0064] Different methods have been developed to combat enzymatic polysorbate degradation in host-cell formulations, but none of them have been decisive. Alternate surfactants without an ester bond are rare, with poloxamer 188 being the only alternative used in only 27% of all formulations (Strickley, et al. 2021. A review of Formulations of Commercially Available Antibodies. Journal of pharmaceutical sciences, 110(7), 2590–2608.e56). This is because poloxamer 188 is generally less effective than polysorbates because they are not hydrophobic enough to protect proteins that are vulnerable to aggregation. (Kim, et al. 2014. Modulation of protein adsorption by poloxamer 188 in relation to polysorbates 80 and 20 at solid surfaces. Journal of pharmaceutical sciences, 103(4), 1043–1049; Yi, et al. 2020). Cell line engineering via CRISPR technology is used to eliminate or reduce the number of HCPs produced during host-cell culture, but this process is

time consuming, expensive, and only applies to the HCP that was edited. Attempts to filter out these HCPs using microfiltration have been tried but can only be applied to a few proteins that meet the size qualifications (Tuameh, et al. 2023. Methods for addressing host cell protein impurities in biopharmaceutical product development. Biotechnology journal, 18(3), e2200115.). Nevertheless, when polysorbate degradation is studied, the consensus is always the same: if the polysorbate is hydrolyzed, its protective abilities disappear, leaving only aggregated proteins in its wake (Dwivedi, et al., Acidic and alkaline hydrolysis of polysorbates under aqueous conditions: Towards understanding polysorbate degradation in biopharmaceutical formulations. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences, 144, 105211; Li, et al. 2021. Profiling Active Enzymes for Polysorbate Degradation in Biotherapeutics by Activity-Based Protein Profiling. Analytical chemistry, 93(23), 8161–8169; Yi, et al. 2020).

[0065] The inventions advantageously employs the hydrolyzed polysorbates (PS20 and PS80), polyoxyethylene sorbitan (POES, such as isolated POES), and/or sPOES, optionally in combination with one or more polysorbate types for effective protection of proteins from damage due to agitation, such as agitation-mediated aggregation. The inventions further provide protein formulations, including antibodies, such as mAb formulations, with enzyme-hydrolyzed polysorbate that are stable upon agitation.

[0066] **Figure 1A-1D** depict molecular structures of commonly used excipients polysorbate 80 (PS80), polysorbate 20 (PS20), Poloxamer 188, and polyethylene glycol-3350 (PEG-3350), respectively. The solution that does not arise lies in the hydrolyzed polysorbates' remains: the hydrophilic heads of the PS80 and PS20. The heads of the polysorbates for both PS80 and PS20 are known as polyoxyethlene sorbitan (POES) and serve as the polar part of the surfactant. **Figure 1E** illustrates hydrophobic tail and hydrophilic head of a surfactant (polysorbate). When polysorbates are made, the POES or synthetic POES (sPOES) is esterified with the FFAs. Meanwhile, the tails of the PS80 and PS20, known as oleic acid and lauric acid respectively, serve as the non-polar part of the surfactant.

[0067] The POES (or sPOES) in the polysorbate can still provide adequate protection against protein aggregation. The protective qualities of POES were tested because of its structural similarities to poloxamer 188. For example, POES and Poloxamer 188 contain polyoxyethylene, which serves as their hydrophilic part. The non-polar tails, free fatty acids,

of the polysorbate were left out. The hydrophilic head of the polysorbate, POES, alone can still be effective in protecting proteins during agitation. These findings are based on studies conducted with agitation on several monoclonal antibodies (mAbs) in the presence of polysorbates (PS), hydrolyzed PS, isolated POES or sPOES.

[0068] The studies described herein show that hydrolytic enzymes such as lipase within host-cell protein culture are no longer a roadblock in protein drug development. The inventions provide that controlling these enzymes by adding additional polysorbate to protein formulations is no longer necessary. Even if HCPs hydrolyze proteins in drug products the hydrolyzed PS still retains its protective effects meaning that the quality of the drug is still protected.

Hydrolyzed PS80/PS20

[0069] Hydrolyzed polysorbates (hPS) contain POES and contaminant tails (for example, FFAs). Upon agitation FFAs increase HMW% and generate insoluble particles that are detectable by OD405. One issue that arises with the hPS is flocculant or insoluble particle formation in the drug product created by the FFAs. Oleic acid of PS80 usually does not present an issue because its melting point is quite low (17° C), so when injected at room temperature, there is not much of particle formation. However, because multi-compendial grade Super-Refined PS80 produced by Croda contains only about 60% PS80 with oleic acid, the other free fatty acids within the formulations may be an issue (Wuchner, et al., 2022; Patel , et al. 2019: U. S. Patent Application Publication No. 2019/0083618A1).

[0070] In contrast, lauric acid of PS20 has proven to be a greater issue because its melting point is much higher than room temperature (such as 43°C), which can cause particle formation easily (Patel, *et al.* 2019). Furthermore, the other free fatty acid esters within the PS20 only worsen particle formation when hydrolyzed. This means that when formulations involving PS20 are degraded by enzymatic hydrolysis or otherwise would most likely fail the mass spectrometry needed to quality it under the USP specifications for particle formation (Doshi, *et al.* 2015. Understanding Particle Formation: Solubility of Free Fatty Acids as Polysorbate 20 Degradation Byproducts in Therapeutic Monoclonal Antibody Formulations. *Molecular pharmaceutics*, 12(11), 3792–3804; USP).

Polyoxyethlene Sorbitan (POES) or synthetic POES (sPOES)

[0071] The isolated POES or sPOES performed better than the samples with both POES and oleic acid in preventing protein aggregation since they do not possess any fatty acids therefore, are unable to contribute to flocculant or particle formation. Most importantly, POES does not possess an ester, meaning that they are unable to be hydrolyzed by any means, making them the best candidate to replace PS in biopharmaceutical drug formulations.

[0072] POES or sPOES is expected to be safe when used subcutaneously or intravenously since PS80 and PS20 have been already used widely. Hydrolytic enzymes such as lipases and esterases are among the 200 enzymes in the human body, meaning that when polysorbates are injected into the body, they are already being hydrolyzed, releasing both POES and FFA (Li, et al. 2021. Profiling Active Enzymes for Polysorbate Degradation in Biotherapeutics by Activity-Based Protein Profiling. Analytical chemistry, 93(23), 8161–8169.). Therefore, for use in subcutaneous or intravenous drug products, POES retains the same safety level as regular polysorbate which already has been proven to be biocompatible (Sun et al. 2017. Component-based biocompatibility and safety evaluation of polysorbate 80. RSC Advances, 7, 15127-15138).

[0073] The inventions are amenable to use with a wide variety of Fc-containing proteins and other proteins. The inventions can be employed in the production of biological and pharmaceutical products. For example, for antibodies, the inventions are amendable for research and production use for diagnostics and therapeutics based upon all major antibody classes, namely IgG, IgA, IgM, IgD and IgE. IgG is a preferred class, such as IgG1 (including IgG1λ and IgG1κ), IgG2 and IgG4. An abbreviated list of exemplary antibodies to be produced according to the inventions include Alirocumab, Atoltivimab, Maftivimab, Odesivimab, Odesivivmab-ebgn, Casirivimab, Imdevimab, Cemiplimab, Cemplimab-rwlc, Davutamig, Dupilumab, Evinacumab, Evinacumab-dgnb, Fasimumab, Nesvacumab, Trevogrumab, Rinucumab and Sarilumab. A longer list of exemplary antibodies to be produced according to the inventions include, but are not limited to, Abciximab, Adalimumab, Adalimumab-atto, Ado-trastuzumab, Alemtuzumab, Alirocumab, Atezolizumab, Avelumab, Basiliximab, Belimumab, Benralizumab, Bevacizumab, Bezlotoxumab, Blinatumomab, Brentuximab vedotin, Brodalumab, Canakinumab, Capromab pendetide, Certolizumab pegol, Cemiplimab, Cetuximab, Denosumab, Dinutuximab, Dupilumab, Durvalumab, Eculizumab, Elotuzumab, Emicizumab-kxwh, Emtansinealirocumab, Evinacumab, Evolocumab, Fianlimab, Fasinumab, Garetosmab,

Golimumab, Guselkumab, Ibritumomab tiuxetan, Idarucizumab, Infliximab, Infliximab-abda, Infliximab-dyyb, Ipilimumab, Itepekimab, Ixekizumab, Linvoseltamab, Mepolizumab, Mibavademab, Necitumumab, Nesvacumab, Nezastomig, Nivolumab, Obiltoxaximab, Obinutuzumab, Odronextamab, Ocrelizumab, Oatumumab, Olaratumab, Omalizumab, Panitumumab, Pembrolizumab, Pertuzumab, Pozelimab, Ramucirumab, Ranibizumab, Raxibacumab, Reslizumab, Rinucumab, Rituximab, Sarilumab, Secukinumab, Siltuximab, Tocilizumab, Tocilizumab, Trastuzumab, Trevogrumab, Ubamatamab, Ustekinumab, and Vedolizumab, and Vonsetamig. Antibodies can include a human antibody, a humanized antibody, a chimeric antibody, a monoclonal antibody, a multispecific antibody, a bispecific antibody, an antigen binding antibody fragment, a single chain antibody, a diabody, triabody or tetrabody, a Fab fragment or a F(ab')2 fragment, an IgD antibody, an IgE antibody, an IgM antibody, an IgG antibody, an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, or an IgG4 antibody. The antibody can be an IgG1 antibody. The antibody can be an IgG2 antibody. The antibody can be an IgG3 antibody. The antibody can be an IgG4 antibody. The antibody can be a chimeric IgG2/IgG4 antibody. The antibody can be a chimeric IgG2/IgG1 antibody. The antibody can be a chimeric IgG2/IgG1/IgG4 antibody.

[0074] In addition to the antibodies described in the Examples and Figures, the antibody can be an anti-Programmed Cell Death 1 antibody (e.g. an anti-PD1 antibody as described in U.S. Pat. Appln. Pub. No. US2015/0203579A1), an anti-Programmed Cell Death Ligand-1 (e.g. an anti-PD-L1 antibody as described in in U.S. Pat. Appln. Pub. No. US2015/0203580A1), an anti-Dll4 antibody, an anti-Angiopoetin-2 antibody (e.g. an anti-ANG2 antibody as described in U.S. Pat. No. 9,402,898), an anti- Angiopoetin-Like 3 antibody (e.g. an antiAngPtl3 antibody as described in U.S. Pat. No. 9,018,356), an anti-platelet derived growth factor receptor antibody (e.g. an anti-PDGFR antibody as described in U.S. Pat. No. 9,265,827), an anti-Erb3 antibody, an anti- Prolactin Receptor antibody (e.g. anti-PRLR antibody as described in U.S. Pat. No. 9,302,015), an anti-Complement 5 antibody (e.g. an 25 anti-C5 antibody as described in U.S. Pat. Appln. Pub. No US2015/0313194A1), an anti-TNF antibody, an anti-epidermal growth factor receptor antibody (e.g. an anti-EGFR antibody as described in U.S. Pat. No. 9,132,192 or an anti-EGFRvIII antibody as described in U.S. Pat. Appln. Pub. No. US2015/0259423A1), an anti-Proprotein Convertase Subtilisin Kexin-9 antibody (e.g. an anti-PCSK9 antibody as described in U.S. Pat. No. 8,062,640 or U.S. Pat. Appln. Pub. No. US2014/0044730A1), an anti-Growth And Differentiation Factor-8 antibody (e.g. an anti-GDF8 antibody, also known as anti-myostatin antibody, as described in U.S. Pat

Nos. 8,871,209 or 9,260,515), an anti-Glucagon Receptor (e.g. anti-GCGR antibody as described in U.S. Pat. Appln. Pub. Nos. US2015/0337045A1 or US2016/0075778A1), an anti-VEGF antibody, an anti-IL1R antibody, an interleukin 4 receptor antibody (e.g an antiIL4R antibody as described in U.S. Pat. Appln. Pub. No. US2014/0271681A1 or U.S. Pat Nos. 8,735,095 or 8,945,559), an anti-interleukin 6 receptor antibody (e.g. an anti-IL6R antibody as described in U.S. Pat. Nos. 7,582,298, 8,043,617 or 9,173,880), an anti-IL1 antibody, an anti-IL2 antibody, an anti-IL3 antibody, an anti-IL4 antibody, an anti-IL5 antibody, an anti-IL6 antibody, an anti-IL7 antibody, an anti-interleukin 33 (e.g. anti- IL33 antibody as described in U.S. Pat. Appln. Pub. Nos. US2014/0271658A1 or US2014/0271642A1), an anti-Respiratory syncytial virus antibody (e.g. anti-RSV antibody as described in U.S. Pat. Appln. Pub. No. US2014/0271653A1), an anti-Cluster of differentiation 3 (e.g. an anti-CD3 antibody, as described in U.S. Pat. Appln. Pub. Nos. US2014/0088295A1 and US20150266966A1, and in U.S. Application No. 62/222,605), an anti- Cluster of differentiation 20 (e.g. an anti-CD20 antibody as described in U.S. Pat. Appln. Pub. Nos. US2014/0088295A1 and US20150266966A1, and in U.S. Pat. No. 7,879,984), an anti-CD19 antibody, an anti-CD28 antibody, an anti- Cluster of Differentiation 48 (e.g. anti-CD48 antibody as described in U.S. Pat. No. 9,228,014), anti-Fel d1 antibodies (e.g. as described in U.S. Pat. No. 9,079,948), an anti-Middle East Respiratory Syndrome virus (e.g. an anti-MERS antibody as described in U.S. Pat. Appln. Pub. No. US2015/0337029A1), an anti-Ebola virus antibody (e.g. as described in U.S. Pat. Appln. Pub. No. US2016/0215040), an anti-Zika virus antibody, an anti-Lymphocyte Activation Gene 3 antibody (e.g. an anti-LAG3 antibody, or an anti-CD223 antibody), an anti-Nerve Growth Factor antibody (e.g. an anti-NGF antibody as described in U.S. Pat. Appln. Pub. No. US2016/0017029 and U.S. Pat. Nos. 8,309,088 and 9,353,176) and an anti-Activin A antibody. The bispecific antibody can be an anti-CD3 x anti-CD20 bispecific antibody (as described in U.S. Pat. Appln. Pub. Nos. US2014/0088295A1 and US20150266966A1), an anti-CD3 x anti-Mucin 16 bispecific antibody (e.g., an anti-CD3 x anti-Muc16 bispecific antibody), and an anti-CD3 x anti- Prostate-specific membrane antigen bispecific antibody (e.g., an anti-CD3 x anti-PSMA bispecific antibody), an anti-factor XI antibody, anti-EGFR x anti-CD28 bispecific antibody, a bispecific antibody that binds to different epitopes on MET, an anti-BCMA (B cell maturation antigen) x anti-CD3 bispecific antibody, an anti-PSMA x anti-CD28 bispecific antibody, an anti-PD-L1 antibody x anti-IL2Ra, an anti-MET x anti-MET (two distinct epitopes of Mesenchymal Epithelial Transition (MET)) bispecific

antibody, an anti-CD28 x anti-Mucin 16 bispecific antibody (e.g., an anti-CD28 x anti-Muc16 bispecific antibody), an anti-CD22 x anti-CD28 bispecific antibody, an anti-epidermal growth factor receptor antibody x anti-CD28 bispecific antibody (e.g. an anti-EGFR x anti-CD28 bispecific antibody), a bispecific antibody targeting PD1-IL2Ra, an antibody targeting NPR1, a bispecific antibody targeting BCMA and CD3, an antibody targeting IL2Rg, an antibody targeting TMPRSS6, an antibody targeting IL-6R. an antibody targeting myostatin (GDF8), and antibodies targeting Bet v 1. See also U.S. Patent Publication No. US 2019/0285580 A1.

[0075] The inventions also are amenable to the production of other molecules, including fusion proteins. Preferred fusion proteins include Receptor-Fc-fusion proteins, such as Trap proteins. the protein of interest is a recombinant protein that contains an Fc moiety and another domain, (e.g., an Fc-fusion protein). The Fc-fusion protein can be a receptor Fc-fusion protein, which contains one or more extracellular domain(s) of a receptor coupled to an Fc moiety. The Fc moiety can comprise a hinge region followed by a CH2 and CH3 domain of an IgG. The receptor Fc-fusion protein can contain two or more distinct receptor chains that bind to either a single ligand or multiple ligands. For example, an Fc-fusion protein is a TRAP protein, such as for example an IL-1 trap (e.g., rilonacept, which contains the IL-1RAcP ligand binding region fused to the Il-1R1 extracellular region fused to Fc of hIgG1; see U.S. Pat. No. 6,927,044, or a VEGF trap (e.g., aflibercept (including HD) and ziv-aflibercept), which contain the Ig domain 2 of the VEGF receptor Flt1 fused to the Ig domain 3 of the VEGF receptor Flk1 fused to Fc of hIgG1; see U.S. Pat. Nos. 7,087,411 and 7,279,159). The Fc-fusion protein can be a ScFv-Fc-fusion protein, which contains one or more of one or more antigen binding domain(s), such as a variable heavy chain fragment and a variable light chain fragment, of an antibody coupled to an Fc moiety.

[0076] Other proteins lacking Fc portions, such as recombinantly produced enzymes and mini-traps, also can be made according to the inventions . Mini-traps are trap proteins that use a multimerizing component (MC) instead of an Fc portion, and are disclosed in U.S. Patent Nos. 7,279,159 and 7,087,411. Derivatives, components, domains, chains and fragments of the above also are included.

Surfactants

[0077] The protein formulations of the present invention include one or more surfactants or surfactant replacement (i.e. POES (such as isolated POES) or sPOES), optionally in combination with one or more polysorbate types.

[0078] The protein formulations of the present inventions contain a stabilizing concentration of a pharmaceutically acceptable non-ionic surfactant. Pharmaceutically acceptable non-ionic surfactants that may be used in the formulations discussed herein include, without limitation, hydrolyzed polysorbate 80 (hPS80), hydrolyzed polysorbate 20 (hPS20), isolated Polyoxyethlene Sorbitan (POES), sPOES, polysorbate 80 (Tween 80; PS80), polysorbate 20 (Tween 20; PS20) Polysorbates 40 and 60 also can be utilized. Various poloxamers (e.g., poloxamer 188), or mixtures thereof with other surfactants can be utilized. Mixtures of surfactants can be employed in accordance with the teachings disclosed herein.

[0079] "Polysorbate types" are non-ionic surfactants, and include polysorbate 20 (PS20), polysorbate 40 (PS40), polysorbate 60 (PS60) and polysorbate 80 (PS80).

[0080] The protein formulations of the present inventions can comprise from about 0.001% (w/v) to about 0.3% (w/v) of one or more non-ionic surfactants or surfactant replacement (e.g.., POES or sPOES) or in combination with one or more polysorbate types. The protein formulations of the present inventions can comprise non-ionic surfactant(s) or surfactant replacement (e.g., POES or sPOES) or in combination with one or more polysorbate types from about 0.001 % (w/v) to about 0.002 % (w/v), about 0.002 % (w/v) to about 0.003 % (w/v), about 0.003 % (w/v) to about 0.004 % (w/v), about 0.004 % (w/v) to about 0.005 % (w/v), about 0.005 % (w/v) to about 0.006 % (w/v), about 0.006 % (w/v) to about 0.007 % (w/v), about 0.007 % (w/v) to about 0.008 % (w/v), about 0.008 % (w/v) to about 0.009 % (w/v), about 0.009 % (w/v) to about 0.01 % (w/v), 0.01 % (w/v) to about 0.02 % (w/v), 0.02 % (w/v) to about 0.03 % (w/v), 0.03 % (w/v) to about 0.04 % (w/v), 0.04 % (w/v) to about 0.05 % (w/v), 0.05 % (w/v) to about 0.06 % (w/v), 0.06 % (w/v) to about 0.07 % (w/v), 0.07 % (w/v) to about 0.08 % (w/v), 0.08 % (w/v) to about 0.09 % (w/v), 0.09 % (w/v) to about 0.10 % (w/v), 0.10 % (w/v) to about 0.11 % (w/v), 0.11 % (w/v) to about 0.12 % (w/v), 0.12 % (w/v) to about 0.13 % (w/v), 0.13 % (w/v) to about 0.14 % (w/v), 0.14 % (w/v) to about 0.15 % (w/v), 0.15 % (w/v) to about 0.16 % (w/v), 0.16 % (w/v) to about 0.17 % (w/v), 0.17 % (w/v) to about 0.18 %

(w/v), 0.18 % (w/v) to about 0.19 % (w/v), 0.19 % (w/v) to about 0.20 % (w/v), 0.20 % (w/v) to about 0.21 % (w/v), 0.21 % (w/v) to about 0.22 % (w/v), 0.22 % (w/v) to about 0.23 % (w/v), 0.23 % (w/v) to about 0.24 % (w/v), 0.24 % (w/v) to about 0.25 % (w/v), 0.25 % (w/v) to about 0.26 % (w/v), 0.26 % (w/v) to about 0.27 % (w/v), 0.27 % (w/v) to about 0.28 % (w/v), 0.28 % (w/v) to about 0.29 % (w/v), or 0.29 % (w/v) to about 0.30 % (w/v) or any integer therebetween. The formulations can comprise about 0.0015% (w/v), about 0.0025% (w/v), about 0.0035% (w/v), about 0.0045% (w/v), about 0.0055% (w/v), about 0.0065% (w/v), about 0.0075% (w/v), about 0.0085% (w/v), or about 0.0095% (w/v) non-ionic surfactant. The protein formulations of the present inventions comprise about 0.001 % (w/v), about 0.0015 % (w/v), about 0.002 % (w/v), about 0.0025 % (w/v), about 0.003 % (w/v), about 0.0035 % (w/v), about 0.004 % (w/v), about 0.0045 % (w/v), about 0.005 % (w/v), about 0.0055 % (w/v), about 0.006 % (w/v), about 0.0065 % (w/v), about 0.007 % (w/v), about 0.0075 % (w/v), about 0.008 % (w/v), about 0.0085 % (w/v), about 0.009 % (w/v), about 0.0095 % (w/v), about 0.01 % (w/v), about 0.02 % (w/v), about 0.03 % (w/v), about 0.04 % (w/v), about 0.05 % (w/v), about 0.06 % (w/v), about 0.07 % (w/v), about 0.08 % (w/v), about 0.09 % (w/v), about 0.1 % (w/v), about 0.11 % (w/v), about 0.12 % (w/v), about 0.13 % (w/v), about 0.14 % (w/v), about 0.15 % (w/v), about 0.16 % (w/v), about 0.17 % (w/v), about 0.18 % (w/v), about 0.19 % (w/v), about 0.2 % (w/v), about 0.21 % (w/v), about 0.22 % (w/v), about 0.23 % (w/v), about 0.24 % (w/v), about 0.25 % (w/v), about 0.26 % (w/v), about 0.27 % (w/v), about 0.28 % (w/v), about 0.29 % (w/v), or about 0.3 % (w/v) non-ionic surfactant. The protein formulations of the present inventions comprise 0.20% w/v ± 0.01% w/v non-ionic surfactant.

[0081] The protein formulations of the present inventions can comprise about 0.01% (w/v), about 0.015% (w/v), about 0.02% (w/v), about 0.025% (w/v), about 0.03% (w/v), about 0.035% (w/v), about 0.04% (w/v), about 0.045% (w/v), about 0.05% (w/v), about 0.055% (w/v), about 0.06% (w/v), about 0.065% (w/v), about 0.07% (w/v), about 0.075% (w/v), about 0.08% (w/v), about 0.085% (w/v), about 0.09% (w/v), about 0.095% (w/v), about 0.10% (w/v), about 0.15% (w/v), about 0.20% (w/v), about 0.25% (w/v), or about 0.30% (w/v) hydrolyzed polysorbate 80 (hPS80), hydrolyzed polysorbate 20 (hPS20), and/or isolated Polyoxyethlene Sorbitan (POES) and/or sPOES. The protein formulations of the present inventions can comprise 0.20% w/v ± 0.01% w/v hydrolyzed polysorbate 80 (hPS80), hydrolyzed polysorbate 20 (hPS20), and/or isolated Polyoxyethlene Sorbitan (POES) and/or sPOES, for example.

[0082] The protein formulations of the present inventions can comprise a combination of Polyoxyethlene Sorbitan (POES) and/or sPOES and polysorbate 80 (Tween 80; PS80) and/or polysorbate 20 (Tween 20; PS20). The protein formulations can comprise from about 0.001% (w/v) to about 0.3% (w/v) POES and/or sPOES, optionally in combination with about 0.001% (w/v) to about 0.3% (w/v) of PS80 and/or PS20.

[0083] The protein formulations of the present inventions can comprise from about 0.001 % (w/v) to about 0.002 % (w/v), about 0.002 % (w/v) to about 0.003 % (w/v), about 0.003 % (w/v) to about 0.004 % (w/v), about 0.004 % (w/v) to about 0.005 % (w/v), about 0.005 % (w/v) to about 0.006 % (w/v), about 0.006 % (w/v) to about 0.007 % (w/v), about 0.007 % (w/v) to about 0.008 % (w/v), about 0.008 % (w/v) to about 0.009 % (w/v), about 0.009 % (w/v) to about 0.01 % (w/v), 0.01 % (w/v) to about 0.02 % (w/v), 0.02 % (w/v) to about 0.03 % (w/v), 0.03 % (w/v) to about 0.04 % (w/v), 0.04 % (w/v) to about 0.05 % (w/v), 0.05 % (w/v) to about 0.06 % (w/v), 0.06 % (w/v) to about 0.07 % (w/v), 0.07 % (w/v) to about 0.08 % (w/v), 0.08 % (w/v) to about 0.09 % (w/v), 0.09 % (w/v) to about 0.10 % (w/v), 0.10 % (w/v) to about 0.11 % (w/v), 0.11 % (w/v) to about 0.12 % (w/v), 0.12 % (w/v) to about 0.13 % (w/v), 0.13 % (w/v) to about 0.14 % (w/v), 0.14 % (w/v) to about 0.15 % (w/v), 0.15 % (w/v) to about 0.16 % (w/v), 0.16 % (w/v) to about 0.17 % (w/v), 0.17 % (w/v) to about 0.18 % (w/v), 0.18 % (w/v) to about 0.19 % (w/v), 0.19 % (w/v) to about 0.20 % (w/v), 0.20 % (w/v) to about 0.21 % (w/v), 0.21 % (w/v) to about 0.22 % (w/v), 0.22 % (w/v) to about 0.23 % (w/v), 0.23 % (w/v) to about 0.24 % (w/v), 0.24 % (w/v) to about 0.25 % (w/v), 0.25 % (w/v) to about 0.26 % (w/v), 0.26 % (w/v) to about 0.27 % (w/v), 0.27 % (w/v) to about 0.28 % (w/v), 0.28 % (w/v) to about 0.29 % (w/v), or 0.29 % (w/v) to about 0.30 % (w/v) POES and/or sPOES in combination with any of about 0.001 % (w/v) to about 0.002 % (w/v), about 0.002 % (w/v) to about 0.003 % (w/v), about 0.003 % (w/v) to about 0.004 % (w/v), about 0.004 % (w/v) to about 0.005 % (w/v), about 0.005 % (w/v) to about 0.006 % (w/v), about 0.006 % (w/v) to about 0.007 % (w/v), about 0.007 % (w/v) to about 0.008 % (w/v), about 0.008 % (w/v) to about 0.009 % (w/v), about 0.009 % (w/v) to about 0.01 % (w/v), 0.01 % (w/v) to about 0.02 % (w/v), 0.02 % (w/v) to about 0.03 % (w/v), 0.03 % (w/v) to about 0.04 % (w/v), 0.04 % (w/v) to about 0.05 % (w/v), 0.05 % (w/v) to about 0.06 % (w/v), 0.06 % (w/v) to about 0.07 % (w/v), 0.07 % (w/v) to about 0.08 % (w/v), 0.08 % (w/v) to about 0.09 % (w/v), 0.09 % (w/v) to about 0.10 % (w/v), 0.10 % (w/v) to about 0.11 % (w/v), 0.11 % (w/v) to about 0.12 % (w/v), 0.12 % (w/v) to about 0.13 % (w/v), 0.13 % (w/v) to about 0.14 % (w/v), 0.14 % (w/v) to about 0.15 % (w/v), 0.15 % (w/v) to about 0.16 %

(w/v), 0.16 % (w/v) to about 0.17 % (w/v), 0.17 % (w/v) to about 0.18 % (w/v), 0.18 % (w/v) to about 0.19 % (w/v), 0.19 % (w/v) to about 0.20 % (w/v), 0.20 % (w/v) to about 0.21 % (w/v), 0.21 % (w/v) to about 0.22 % (w/v), 0.22 % (w/v) to about 0.23 % (w/v), 0.23 % (w/v) to about 0.24 % (w/v), 0.24 % (w/v) to about 0.25 % (w/v), 0.25 % (w/v) to about 0.26 % (w/v), 0.26 % (w/v) to about 0.27 % (w/v), 0.27 % (w/v) to about 0.28 % (w/v), 0.28 % (w/v) to about 0.29 % (w/v), or 0.29 % (w/v) to about 0.30 % (w/v) of PS80 or PS20. The protein formulations can comprise a combination of a higher range of POES and/or sPOES in combination with any of a lower range of PS80 or PS20, and *vice versa*.

[0084] The compositions can comprise about 0.0015% (w/v), about 0.0025% (w/v), about 0.0035% (w/v), about 0.0045% (w/v), about 0.0055% (w/v), about 0.0065% (w/v), about 0.0075% (w/v), about 0.0085% (w/v), or about 0.0095% (w/v) POES and/or sPOES in combination with any of about 0.0015% (w/v), about 0.0025% (w/v), about 0.0035% (w/v), about 0.0045% (w/v), about 0.0055% (w/v), about 0.0065% (w/v), about 0.0075% (w/v), about 0.0085% (w/v), or about 0.0095% (w/v) of PS80 or PS20. The protein formulations of the present inventions can comprise about 0.001 % (w/v), about 0.0015 % (w/v), about 0.002 % (w/v), about 0.0025 % (w/v), about 0.003 % (w/v), about 0.0035 % (w/v), about 0.004 % (w/v), about 0.0045 % (w/v), about 0.005 % (w/v), about 0.0055 % (w/v), about 0.006 % (w/v), about 0.0065 % (w/v), about 0.007 % (w/v), about 0.0075 % (w/v), about 0.008 % (w/v), about 0.0085 % (w/v), about 0.009 % (w/v), about 0.0095 % (w/v), about 0.01 % (w/v), about 0.02 % (w/v), about 0.03 % (w/v), about 0.04 % (w/v), about 0.05 % (w/v), about 0.06 % (w/v), about 0.07 % (w/v), about 0.08 % (w/v), about 0.09 % (w/v), about 0.1 % (w/v), about 0.11 % (w/v), about 0.12 % (w/v), about 0.13 % (w/v), about 0.14 % (w/v), about 0.15 % (w/v), about 0.16 % (w/v), about 0.17 % (w/v), about 0.18 % (w/v), about 0.19 % (w/v), about 0.2 % (w/v), about 0.21 % (w/v), about 0.22 % (w/v), about 0.23 % (w/v), about 0.24 % (w/v), about 0.25 % (w/v), about 0.26 % (w/v), about 0.27 % (w/v), about 0.28 % (w/v), about 0.29 % (w/v), about 0.3 % (w/v) POES and/or sPOES in combination with any of about 0.001 % (w/v), about 0.0015 % (w/v), about 0.002 % (w/v), about 0.0025 % (w/v), about 0.003 % (w/v), about 0.0035 % (w/v), about 0.004 % (w/v), about 0.0045 % (w/v), about 0.005 % (w/v), about 0.0055 % (w/v), about 0.006 % (w/v), about 0.0065 % (w/v), about 0.007 % (w/v), about 0.0075 % (w/v), about 0.008 % (w/v), about 0.0085 % (w/v), about 0.009 % (w/v), about 0.0095 % (w/v), about 0.01 % (w/v), about 0.02 % (w/v), about 0.03 % (w/v), about 0.04 % (w/v), about 0.05 % (w/v), about 0.06 % (w/v), about 0.07 % (w/v), about

0.08 % (w/v), about 0.09 % (w/v), about 0.1 % (w/v), about 0.11 % (w/v), about 0.12 % (w/v), about 0.13 % (w/v), about 0.14 % (w/v), about 0.15 % (w/v), about 0.16 % (w/v), about 0.17 % (w/v), about 0.18 % (w/v), about 0.19 % (w/v), about 0.2 % (w/v), about 0.21 % (w/v), about 0.22 % (w/v), about 0.23 % (w/v), about 0.24 % (w/v), about 0.25 % (w/v), about 0.26 % (w/v), about 0.27 % (w/v), about 0.28 % (w/v), about 0.29 % (w/v), about 0.3 % (w/v) of PS80 or PS20.

[0085] The protein formulations can comprise from about 0.001% (w/v) to about 0.30% (w/v) POES and/or sPOES in combination with about 0.001% (w/v) to about 0.10% (w/v) of PS80 and/or PS20. The protein formulations can comprise from about 0.01% (w/v) POES and/or sPOES in combination with about 0.005% (w/v) or about 0.01% (w/v) of PS80 and/or PS20. The protein formulations can comprise from about 0.05% (w/v) POES and/or sPOES in combination with about 0.05% (w/v) or about 0.01% (w/v) of PS80 and/or PS20.

[0086] The protein formulations of the present inventions can comprise about 0.001% (w/v) to about 0.01% (w/v) polysorbate 80. The protein formulations of the present inventions can comprise about 0.001% (w/v) to about 0.01% (w/v) poloxamer 188. The protein formulations of the present inventions can comprise about 0.001% (w/v), about 0.0015% (w/v), about 0.002% (w/v), about 0.0025% (w/v), about 0.003% (w/v), about 0.0035% (w/v), about 0.004% (w/v), about 0.0045% (w/v), about 0.005% (w/v), about 0.0055% (w/v), about 0.006% (w/v), about 0.0065% (w/v), about 0.007% (w/v), about 0.0075% (w/v), about 0.008% (w/v), about 0.0085% (w/v), about 0.009% (w/v), about 0.0095% (w/v), or about 0.01% (w/v) polysorbate 80. The protein formulations of the present inventions can comprise about 0.001% (w/v), about 0.0015% (w/v), about 0.002% (w/v), about 0.0025% (w/v), about 0.003% (w/v), about 0.0035% (w/v), about 0.004% (w/v), about 0.0045% (w/v), about 0.005% (w/v), about 0.0055% (w/v), about 0.006% (w/v), about 0.0065% (w/v), about 0.007% (w/v), about 0.0075% (w/v), about 0.008% (w/v), about 0.0085% (w/v), about 0.009% (w/v), about 0.0095% (w/v), or about 0.01% (w/v) poloxamer 188. The protein formulations of the present inventions can comprise about 0.005% w/v ± 0.001% w/v polysorbate 80 or about 0.005% w/v ± 0.001% w/v poloxamer 188.

Buffers and pH

[0087] The protein formulations of the present inventions include pharmaceutically acceptable buffering agents. In some cases, the buffering agents include, without limitation, phosphate buffers, histidine buffers, sodium citrate buffers, HEPES buffers, Tris buffers,

Bicine buffers, glycine buffers, N-glycylglycine buffers, sodium acetate buffers, sodium carbonate buffers, glycyl glycine buffers, lysine buffers, arginine buffers, sodium phosphate buffers, and/or mixtures thereof. The buffering agent can be a Histidine buffer, a Phosphate buffer (e.g., a sodium phosphate buffer) or a Tris buffer.

[0088] The protein formulations of the present inventions can comprise a buffering agent of about 1 to about 30, about 1 mM to about 20 mM, about 1 mM to about 10 mM, about 1 mM to about 5 mM, about 5 mM to about 10 mM, about 5 mM to about 15 mM, about 5 mM to about 20 mM, about 10 mM to about 15 mM, about 10 mM to about 20 mM, about 20 mM to about 25 mM, about 25 mM to about 30 mM, about 5 mM to about 25 mM, about 5 to about 30, about 7 mM to about 13 mM, or about 8 mM to about 12 mM, or of any integer therebetween. The formulations can comprise about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM, about 20 mM, about 21 mM, about 22 mM, about 23 mM, about 24 mM, about 25 mM, about 26 mM, about 27 mM, about 28 mM, about 29 mM, or about 30 mM of a buffering agent.

[0089] The protein formulations of the present inventions can comprise Tris buffer of about 1 mM to about 20 mM, about 1 mM to about 10 mM, about 1 mM to about 5 mM, about 5 mM to about 20 mM, about 5 mM to about 10 mM, about 10 mM to about 15 mM, about 10 mM to about 20 mM, about 1 mM to about 20 mM, about 1 mM to about 20 mM, about 5 mM to about 8 mM, about 8 mM to about 12 mM, or 10 mM ± 1 mM, or of any integer therebetween. The formulations can comprise sodium phosphate buffer of about 1 mM to about 20 mM, about 5 mM to about 15 mM, about 8 mM to about 12 mM, or 10 mM ± 1 mM, or of any integer therebetween. The formulations can comprise about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM, or about 20 mM Tris buffer. The formulations can comprise about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM, or about 20 mM sodium phosphate buffer. The formulations can also comprise 10 mM ± 2 mM Tris buffer. The formulations can comprise 10 mM ± 2 mM sodium phosphate buffer.

[0090] The protein formulations of the present inventions can comprise Histidine buffer of about 1 mM to about 40 mM, about 5 mM to about 30 mM, about 20 mM to about 30 mM, about 5 mM to about 10 mM, about 5 mM to about 15 mM, about 5 mM to about 20 mM, about 5 mM to about 25 mM, about 10 mM to about 15 mM, about 10 mM to about 20 mM, about 10 mM to about 25 mM, about 20 mM to about 25 mM, or 10 mM ± 1 mM, or of any integer therebetween. The formulations can comprise about 1 mM to about 40 mM, about 1 mM to about 30 mM, about 1 mM to about 20 mM, or 10 mM ± 1 mM Histidine buffer. The formulations can comprise about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM, about 20 mM, about 21 mM, about 22 mM, about 23 mM, about 24 mM, about 25 mM, about 26 mM, about 27 mM, about 28 mM, about 29 mM, about 30 mM, about 31 mM, about 32 mM, about 33 mM, about 34 mM, about 35 mM, about 36 mM, about 37 mM, about 38 mM, about 39 mM, or about 40 mM Histidine buffer.

[0091] The protein formulations of the present inventions have a physiologically compatible pH. The mAb formulations are provided that contain a buffering agent suitable to maintain the formulations at or near a neutral pH.

[0092] The pH of the protein formulations of the present inventions is about 5.0 to about 9.0, about 5.5 to about 8.5, about 5.6 to about 8.0, about 5.7 to about 7.5, about 5.8 to about 7.0, about 5.9 to about 7.0, about 5.0 to about 7.0, about 6.5 to about 8.0, about 6.9 to about 7.7, or about 7.0 to about 7.5. The pH of the formulations is about 6.5 or about 6.6, about 6.7, about 6.8, about 6.9, about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, about 7.8, about 7.9, about 8.0, about 8.1, about 8.2, about 8.3, about 8.4, about 8.5, about 8.6, about 8.7, about 8.8, about 8.9, or about 9.0. The pH of the protein formulations of the present inventions is about 5.5, about 5.6, about 5.7, about 5.8 or about 5.9. The pH of the protein formulations of the present inventions is about 5.7 ± 0.1. The pH of the protein formulations of the present inventions is about 5.7 ± 0.05. The pH of the protein formulations of the present inventions is about 5.9 ± 0.1. The pH of the protein formulations of the present inventions is about 5.9 ± 0.05.

Sugars and Sugar Alcohols

[0093] The protein formulations of the present inventions can include one or more sugars, and are optional.

[0094] Inclusion of one of more sugars (e.g., at between about 1% to about 20%) improves the stability of the liquid formulations of the present inventions. The protein formulations of the present inventions contain from about 1% to about 10% of one or more sugars. Any sugar such as mono-, di-, or polysaccharides, or water-soluble glucans, including for example fructose, glucose, mannose, sorbose, xylose, maltose, lactose, sucrose, dextran, trehalose, pullulan, dextrin, cyclodextrin, soluble starch, hydroxyethyl starch, and carboxymethylcellulose can be used in the formulation. The sugar can be sucrose, trehalose, or a combination thereof.

[0095] The sugars may be used individually or in combination. The sugar, or a combination thereof, is present in the formulations or composition at a concentration of about 1% to about 10% (w/v), about 2% (w/v) to about 8% (w/v), about 2.5% to about 7.5% (w/v), about 3% (w/v) to about 7% (w/v), or about 4% to about 6% (w/v). The protein formulations of the present inventions can comprise about 1.0% (w/v), about 1.1% (w/v), about 1.2% (w/v), about 1.3% (w/v), about 1.4% (w/v), about 1.5% (w/v), about 1.6% (w/v), about 1.7% (w/v), about 1.8% (w/v), about 1.9% (w/v), about 2.0% (w/v), about 2.1% (w/v), about 2.2% (w/v), about 2.3% (w/v), about 2.4% (w/v), about 2.5% (w/v), about 2.6% (w/v), about 2.7% (w/v), about 2.8% (w/v), about 2.9% (w/v), about 3.0% (w/v), about 3.1% (w/v), about 3.2% (w/v), about 3.3% (w/v), about 3.4% (w/v), about 3.5% (w/v), about 3.6% (w/v), about 3.7% (w/v), about 3.8% (w/v), about 3.9% (w/v), about 4.0% (w/v), about 4.1% (w/v), about 4.2% (w/v), about 4.3% (w/v), about 4.4% (w/v), about 4.5% (w/v), about 4.6% (w/v), about 4.7% (w/v), about 4.8% (w/v), about 4.9% (w/v), about 5.0% (w/v), about 5.1% (w/v), about 5.2% (w/v), about 5.3% (w/v), about 5.4% (w/v), about 5.5% (w/v), about 5.6% (w/v), about 5.7% (w/v), about 5.8% (w/v), about 5.9% (w/v), about 6.0% (w/v), about 6.1% (w/v), about 6.2% (w/v), about 6.3% (w/v), about 6.4% (w/v), about 6.5% (w/v), about 6.6% (w/v), about 6.7% (w/v), about 6.8% (w/v), about 6.9% (w/v), about 7.0% (w/v), about 7.1% (w/v), about 7.2% (w/v), about 7.3% (w/v), about 7.4% (w/v), about 7.5% (w/v), about 7.6% (w/v), about 7.7% (w/v), about 7.8% (w/v), about 7.9% (w/v), about 8.0% (w/v), about 8.1% (w/v), about 8.2% (w/v), about 8.3% (w/v), about 8.4% (w/v), about 8.5% (w/v), about 8.6% (w/v), about 8.7% (w/v), about 8.8% (w/v), about 8.9% (w/v), about 9.0% (w/v), about 9.1% (w/v), about 9.2% (w/v), about 9.3% (w/v), about 9.4% (w/v), about 9.5% (w/v), about 9.6% (w/v), about 9.7% (w/v), about 9.8% (w/v), about 19.9% (w/v), or about 10% (w/v) sugar.

[0096] The protein formulations of the present inventions include about 2.5% w/v to about 7.5% w/v sucrose. The formulations contains contain 4% w/v to 6% w/v sucrose. The formulations contains 4.5% w/v ± 0.5% or 5.0% w/v ± 0.5% w/v sucrose.

[0097] Sugar alcohols, also known as polyols, can be used according to the inventions in combination for sugars or as a substitute for sugars. Sugar alcohols include Glycerol, Mannitol, Sorbitol, Xylitol, Fucitol, Galactitol, Erythritol, Hydrogenated starch hydrolysates (HSH), Inositol, Iditol, Lactitol, and Maltitol.

Salts

[0098] The protein formulations of the present inventions include one or more pharmaceutically acceptable salts.

[0099] The pharmaceutically acceptable salts include, but are not limited to, metal salts such as sodium, potassium and cesium salts; alkaline earth metal salts such as calcium and magnesium salts; organic amine salts such as triethylamine, guanidine and N-substituted guanidine salts, acetamidine and N-substituted acetamidine, pyridine, picoline, ethanolamine, triethanolamine, dicyclohexylamine, and N,N′-dibenzylethylenediamine salts. Pharmaceutically acceptable salts (of basic nitrogen centers) include, but are not limited to inorganic acid salts such as the hydrochloride, hydrobromide, sulfate, phosphate; organic acid salts such as trifluoroacetate and maleate salts; sulfonates such as methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, camphor sulfonate and naphthalenesulfonate; amino acid salts such as arginate, alaninate, asparginate and glutamate; and carbohydrate salts such as gluconate and galacturonate. Non-limiting examples of pharmaceutically acceptable salts include, without limitation, sodium salts, ammonium salts, potassium salts, calcium salts, and magnesium salts (e.g., sodium, ammonium, potassium, calcium, and magnesium chloride; sodium, ammonium, potassium, calcium and magnesium acetate; sodium, ammonium, potassium, calcium and magnesium citrate; sodium, ammonium, potassium, calcium and magnesium phosphate; sodium, ammonium, potassium, calcium and magnesium fluoride; sodium, ammonium, potassium, calcium and magnesium bromide; and sodium, ammonium, potassium, calcium and magnesium iodide). The pharmaceutically acceptable salt is sodium chloride or arginine hydrochloride (L-arginine hydrochloride).

[00100] The protein formulations of the present inventions can comprise about 0 mM to about 150 mM, about 5 mM to about 150 mM, about 5 mM to about 100 mM, about

5 mM to about 90 mM, about 5 mM to about 80 mM, about 5 mM to about 70 mM, about 5 mM to about 60 mM, about 5 mM to about 50 mM, about 5 mM to about 40 mM, 5 mM to about 30 mM, about 10 mM to about 50 mM, about 15 mM to about 45 mM, about 20 mM to about 40 mM, about 25 mM to about 35 mM, about 30 mM to about 130 mM, about 40 mM to about 120 mM, about 50 mM to about 110 mM, about 60 mM to about 100 mM, about 70 mM to about 90 mM, or about 75 mM to about 85 mM of a pharmaceutically acceptable salt. The protein formulations of the present inventions can comprise about 0 mM, about 5 mM, about 10 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM, about 100 mM, about 105 mM, about 110 mM, about 115 mM, about 120 mM, about 125 mM, about 130 mM, about 135 mM, about 140 mM, about 145 mM, or about 150 mM of a pharmaceutically acceptable salt. The protein formulations of the present inventions can comprise about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM, about 20 mM, about 21 mM, about 22 mM, about 23 mM, about 24 mM, about 25 mM, about 26 mM, about 27 mM, about 28 mM, about 29 mM, about 30 mM, about 31 mM, about 32 mM, about 33 mM, about 34 mM, about 35 mM, about 36 mM, about 37 mM, about 38 mM, about 39 mM, about 40 mM, about 41 mM, about 42 mM, about 43 mM, about 44 mM, about 45 mM, about 46 mM, about 47 mM, about 48 mM, about 49 mM, about 50 mM, about 51 mM, about 52 mM, about 53 mM, about 54 mM, about 55 mM, about 56 mM, about 57 mM, about 58 mM, about 59 mM, about 60 mM, about 61 mM, about 62 mM, about 63 mM, about 64 mM, about 65 mM, about 66 mM, about 67 mM, about 68 mM, about 69 mM, about 70 mM, about 71 mM, about 72 mM, about 73 mM, about 74 mM, about 75 mM, about 76 mM, about 77 mM, about 78 mM, about 79 mM, about 80 mM, about 81 mM, about 82 mM, about 83 mM, about 84 mM, about 85 mM, about 86 mM, about 87 mM, about 88 mM, about 89 mM, about 90 mM, about 91 mM, about 92 mM, about 93 mM, about 94 mM, about 95 mM, about 96 mM, about 97 mM, about 98 mM, about 99 mM, about 100 mM, about 101 mM, about 102 mM, about 103 mM, about 104 mM, or about 105 mM of a pharmaceutically acceptable salt.

[00101] The protein formulations of the present inventions can comprise about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM, about 20 mM, about 21 mM, about 22 mM,

about 23 mM, about 24 mM, about 25 mM, about 26 mM, about 27 mM, about 28 mM, about 29 mM, about 30 mM, about 31 mM, about 32 mM, about 33 mM, about 34 mM, about 35 mM, about 36 mM, about 37 mM, about 38 mM, about 39 mM, about 40 mM, about 41 mM, about 42 mM, about 43 mM, about 44 mM, about 45 mM, about 46 mM, about 47 mM, about 48 mM, about 49 mM, or about 50 mM of arginine hydrochloride. The protein formulations of the present inventions can comprise about 60 mM, about 61 mM, about 62 mM, about 63 mM, about 64 mM, about 65 mM, about 66 mM, about 67 mM, about 68 mM, about 69 mM, about 70 mM, about 71 mM, about 72 mM, about 73 mM, about 74 mM, about 75 mM, about 76 mM, about 77 mM, about 78 mM, about 79 mM, about 80 mM, about 81 mM, about 82 mM, about 83 mM, about 84 mM, about 85 mM, about 86 mM, about 87 mM, about 88 mM, about 89 mM, about 90 mM, about 91 mM, about 92 mM, about 93 mM, about 94 mM, about 95 mM, about 96 mM, about 97 mM, about 98 mM, about 99 mM, or about 100 mM of arginine hydrochloride. The protein formulations of the present inventions can comprise 25 mM ± 5 mM, 35 mM ± 5 mM, 45 mM ± 5 mM, 55 mM ± 5 mM, 60 mM ± 5 mM, 65 mM ± 5 mM, or 75 mM ± 5 mM arginine hydrochloride.

Protein Concentration

[00102] The protein formulations of the present inventions can comprise a protein concentration from about 5 ± 0.75 mg/mL to about 300 ± 50.0 mg/mL, about 10 to 200+ 37.5 mg/mL , or 50 to 150 ± 37.5 mg/mL. Proteins includes antibodies such as monoclonal antibodies, and the like. For example, the antibody concentration in the protein formulations of the present inventions can be about 12.5 mg/mL ± 1.85 mg/mL, about 12.5 mg/mL, about 25 mg/mL ± 3.75 mg/mL, about 25 mg/mL, about 50 mg/mL ± 7.5 mg/mL, about 50 mg/mL, about 100 mg/mL ± 15 mg/mL, about 100 mg/mL, about 150 mg/mL ± 22.5 mg/mL, about 150 mg/mL, about 175 mg/mL ± 26.25 mg/mL, about 175 mg/mL, about 200 mg/mL ± 30 mg/mL, about 200 mg/mL, about 250 ± 37.5 mg/mL, about 250 mg/mL, about 300 ± 50.0 mg/mL, or about 300 mg/mL.

[00103] The protein formulations of the present inventions can comprise a protein concentration from about 10 mg/mL to about 300 mg/mL, about 10 to 290 mg/mL, about 10 to 280 mg/mL, about 10 to 270 mg/mL, about 10 to 260 mg/mL, about 10 to 250 mg/mL, about 10 to 240 mg/mL, about 10 to 230 mg/mL, about 10 to 220 mg/mL, about 10 to 210 mg/mL, about 10 to 200 mg/mL, about 10 to 190 mg/mL, about 10 to 180 mg/mL, about 10 to 170 mg/mL, about 10 to 160 mg/mL, about 10 to 150 mg/mL, about 10 to 140

mg/mL, about 10 to 130 mg/mL, about 10 to 120 mg/mL, about 10 to 110 mg/mL, about 10 to about 100 mg/mL, about 10 to 90 mg/mL, about 10 to 80 mg/mL, about 10 to 70 mg/mL, about 10 to 60 mg/mL, about 10 to 50 mg/mL, about 10 to 40 mg/mL, about 10 to 30 mg/mL, about 10 to 20 mg/mL, about 20 mg/mL to about 300 mg/mL, about 20 to 290 mg/mL, about 20 to 280 mg/mL, about 20 to 270 mg/mL, about 20 to 260 mg/mL, about 20 to 250 mg/mL, about 20 to 240 mg/mL, about 20 to 230 mg/mL, about 20 to 220 mg/mL, about 20 to 210 mg/mL, about 20 to about 200 mg/mL, about 20 to 190 mg/mL, about 20 to 180 mg/mL, about 20 to 170 mg/mL, about 20 to 160 mg/mL, about 20 to 150 mg/mL, about 20 to 140 mg/mL, about 20 to 130 mg/mL, about 20 to 120 mg/mL, about 20 to 110 mg/mL, about 20 to 100 mg/mL, about 20 to 90 mg/mL, about 20 to 80 mg/mL, about 20 to 70 mg/mL, about 20 to 60 mg/mL, about 20 to 50 mg/mL, about 20 to 40 mg/mL, about 20 to 30 mg/mL, about 30 mg/mL to about 300 mg/mL, about 30 to 290 mg/mL, about 30 to 280 mg/mL, about 30 to 270 mg/mL, about 30 to 260 mg/mL, about 30 to 250 mg/mL, about 30 to 240 mg/mL, about 30 to 230 mg/mL, about 30 to 220 mg/mL, about 30 to 210 mg/mL, about 30 to 200 mg/mL, about 30 to 190 mg/mL, about 30 to 180 mg/mL, about 30 to 170 mg/mL, about 30 to 160 mg/mL, about 30 to 150 mg/mL, about 30 to 140 mg/mL, about 30 to 130 mg/mL, about 30 to 120 mg/mL, about 30 to 110 mg/mL, about 30 to 100 mg/mL, about 30 to 90 mg/mL, about 30 to 80 mg/mL, about 30 to 70 mg/mL, about 30 to 60 mg/mL, about 30 to 50 mg/mL, about 30 to 40 mg/mL, about 40 mg/mL to about 300 mg/mL, about 40 to 290 mg/mL, about 40 to 280 mg/mL, about 40 to 270 mg/mL, about 40 to 260 mg/mL, about 40 to 250 mg/mL, about 40 to 240 mg/mL, about 40 to 240 mg/mL, about 40 to 220 mg/mL, about 40 to 210 mg/mL, about 40 to 200 mg/mL, about 40 to 190 mg/mL, about 40 to 180 mg/mL, about 40 to 170 mg/mL, about 40 to 160 mg/mL, about 40 to 150 mg/mL, about 40 to 140 mg/mL, about 40 to 130 mg/mL, about 40 to 120 mg/mL, about 40 to 110 mg/mL, about 40 to 100 mg/mL, about 40 to 90 mg/mL, about 40 to 80 mg/mL, about 40 to 70 mg/mL, about 40 to 60 mg/mL, about 40 to 50 mg/mL, about 50 mg/mL to about 300 mg/mL, about 50 to 290 mg/mL, about 50 to 280 mg/mL, about 50 to 270 mg/mL, about 50 to 260 mg/mL, about 50 to 250 mg/mL, about 50 to 240 mg/mL, about 50 to 240 mg/mL, about 50 to 220 mg/mL, about 50 to 210 mg/mL, about 50 to 200 mg/mL, about 50 to 190 mg/mL, about 50 to 180 mg/mL, about 50 to 170 mg/mL, about 50 to 160 mg/mL, about 50 to 150 mg/mL, about 50 to 140 mg/mL, about 50 to 130 mg/mL, about 50 to 120 mg/mL, about 50 to 110 mg/mL, about 50 to 100 mg/mL, about 50 to 90 mg/mL, about 50 to 80 mg/mL, about 50 to 70 mg/mL, about 50 to 60 mg/mL, about 60 to 280 mg/mL, about 60 to 270 mg/mL, about 60 to

260 mg/mL, about 60 to 250 mg/mL, about 60 to 240 mg/mL, about 60 to 240 mg/mL, about 60 to 220 mg/mL, about 60 to 210 mg/mL, about 60 to 200 mg/mL, about 60 to 190 mg/mL, about 60 to 180 mg/mL, about 60 to 170 mg/mL, about 60 to 160 mg/mL, about 60 to 150 mg/mL, about 60 to 140 mg/mL, about 60 to 130 mg/mL, about 60 to 120 mg/mL, about 60 to 110 mg/mL, about 60 to 100 mg/mL, about 60 to 90 mg/mL, about 60 to 80 mg/mL, about 60 to 70 mg/mL, about 70 to 280 mg/mL, about 70 to 270 mg/mL, about 70 to 260 mg/mL, about 70 to 250 mg/mL, about 70 to 240 mg/mL, about 70 to 240 mg/mL, about 70 to 220 mg/mL, about 70 to 210 mg/mL, about 70 to 200 mg/mL, about 70 to 190 mg/mL, about 70 to 180 mg/mL, about 70 to 170 mg/mL, about 70 to 160 mg/mL, about 70 to 150 mg/mL, about 70 to 140 mg/mL, about 70 to 130 mg/mL, about 70 to 120 mg/mL, about 70 to 110 mg/mL, about 70 to 100 mg/mL, about 70 to 90 mg/mL, about 70 to 80 mg/mL, about 80 to 280 mg/mL, about 80 to 270 mg/mL, about 80 to 260 mg/mL, about 80 to 250 mg/mL, about 80 to 240 mg/mL, about 80 to 240 mg/mL, about 80 to 220 mg/mL, about 80 to 210 mg/mL, about 80 to 200 mg/mL, about 80 to 190 mg/mL, about 80 to 180 mg/mL, about 80 to 170 mg/mL, about 80 to 160 mg/mL, about 80 to 150 mg/mL, about 80 to 140 mg/mL, about 80 to 130 mg/mL, about 80 to 120 mg/mL, about 80 to 110 mg/mL, about 80 to 100 mg/mL, about 80 to 90 mg/mL, about 90 to 280 mg/mL, about 90 to 270 mg/mL, about 90 to 260 mg/mL, about 90 to 250 mg/mL, about 90 to 240 mg/mL, about 90 to 240 mg/mL, about 90 to 220 mg/mL, about 90 to 210 mg/mL, about 90 to 200 mg/mL, about 90 to 190 mg/mL, about 90 to 180 mg/mL, about 90 to 170 mg/mL, about 90 to 160 mg/mL, about 90 to 150 mg/mL, about 90 to 140 mg/mL, about 90 to 130 mg/mL, about 90 to 120 mg/mL, about 90 to 110 mg/mL, about 90 to 100 mg/mL, about 100 to 280 mg/mL, about 100 to 270 mg/mL, about 100 to 260 mg/mL, about 100 to 250 mg/mL, about 100 to 240 mg/mL, about 100 to 240 mg/mL, about 100 to 220 mg/mL, about 100 to 210 mg/mL, about 100 to 200 mg/mL, about 100 to 190 mg/mL, about 100 to 180 mg/mL, about 100 to 170 mg/mL, about 100 to 160 mg/mL, about 100 to 150 mg/mL, about 100 to 140 mg/mL, about 100 to 130 mg/mL, about 100 to 120 mg/mL, about 100 to 110 mg/mL, about 110 to 280 mg/mL, about 110 to 270 mg/mL, about 110 to 260 mg/mL, about 110 to 250 mg/mL, about 110 to 240 mg/mL, about 110 to 240 mg/mL, about 110 to 220 mg/mL, about 110 to 210 mg/mL, about 110 to 200 mg/mL, about 110 to 190 mg/mL, about 110 to 180 mg/mL, about 110 to 170 mg/mL, about 110 to 160 mg/mL, about 110 to 150 mg/mL, about 110 to 140 mg/mL, about 110 to 130 mg/mL, about 110 to 120 mg/mL, about 120 to 280 mg/mL, about 120 to 270 mg/mL, about 120 to 260 mg/mL, about 120 to 250 mg/mL, about 120 to 240 mg/mL, about 120 to

240 mg/mL, about 120 to 220 mg/mL, about 120 to 210 mg/mL, about 120 to 200 mg/mL, about 120 to 190 mg/mL, about 120 to 180 mg/mL, about 120 to 170 mg/mL, about 120 to 160 mg/mL, about 120 to 150 mg/mL, about 120 to 140 mg/mL, about 120 to 130 mg/mL, about 130 to 280 mg/mL, about 130 to 270 mg/mL, about 130 to 260 mg/mL, about 130 to 250 mg/mL, about 130 to 240 mg/mL, about 130 to 240 mg/mL, about 130 to 220 mg/mL, about 130 to 210 mg/mL, about 130 to 200 mg/mL, about 130 to 190 mg/mL, about 130 to 180 mg/mL, about 130 to 170 mg/mL, about 130 to 160 mg/mL, about 130 to 150 mg/mL, about 130 to 140 mg/mL, about 140 to 280 mg/mL, about 140 to 270 mg/mL, about 140 to 260 mg/mL, about 140 to 250 mg/mL, about 140 to 240 mg/mL, about 140 to 240 mg/mL, about 140 to 220 mg/mL, about 140 to 210 mg/mL, about 140 to 200 mg/mL, about 140 to 190 mg/mL, about 140 to 180 mg/mL, about 140 to 170 mg/mL, about 140 to 160 mg/mL, about 140 to 150 mg/mL, about 150 to 280 mg/mL, about 150 to 270 mg/mL, about 150 to 260 mg/mL, about 150 to 250 mg/mL, about 150 to 240 mg/mL, about 150 to 240 mg/mL, about 150 to 220 mg/mL, about 150 to 210 mg/mL, about 150 to 200 mg/mL, about 150 to 190 mg/mL, about 150 to 180 mg/mL, about 150 to 170 mg/mL, about 150 to 160 mg/mL, about 160 to 280 mg/mL, about 160 to 270 mg/mL, about 160 to 260 mg/mL, about 160 to 250 mg/mL, about 160 to 240 mg/mL, about 160 to 240 mg/mL, about 160 to 220 mg/mL, about 160 to 210 mg/mL, about 160 to 200 mg/mL, about 160 to 190 mg/mL, about 160 to 180 mg/mL, about 160 to 170 mg/mL, about 170 to 280 mg/mL, about 170 to 270 mg/mL, about 170 to 260 mg/mL, about 170 to 250 mg/mL, about 170 to 240 mg/mL, about 170 to 240 mg/mL, about 170 to 220 mg/mL, about 170 to 210 mg/mL, about 170 to 200 mg/mL, about 170 to 190 mg/mL, about 170 to 180 mg/mL, about 180 to 280 mg/mL, about 180 to 270 mg/mL, about 180 to 260 mg/mL, about 180 to 250 mg/mL, about 180 to 240 mg/mL, about 180 to 240 mg/mL, about 180 to 220 mg/mL, about 180 to 210 mg/mL, about 180 to 200 mg/mL, about 180 to 190 mg/mL, about 190 to 280 mg/mL, about 190 to 270 mg/mL, about 190 to 260 mg/mL, about 190 to 250 mg/mL, about 190 to 240 mg/mL, about 190 to 240 mg/mL, about 190 to 220 mg/mL, about 190 to 210 mg/mL, about 190 to 200 mg/mL, about 200 to 280 mg/mL, about 200 to 270 mg/mL, about 200 to 260 mg/mL, about 200 to 250 mg/mL, about 200 to 240 mg/mL, about 200 to 240 mg/mL, about 200 to 220 mg/mL, about 200 to 210 mg/mL, about 210 to 280 mg/mL, about 210 to 270 mg/mL, about 210 to 260 mg/mL, about 210 to 250 mg/mL, about 210 to 240 mg/mL, about 210 to 240 mg/mL, about 210 to 220 mg/mL, about 220 to 280 mg/mL, about 220 to 270 mg/mL, about 220 to 260 mg/mL, about 220 to 250 mg/mL, about 220 to 240 mg/mL, about 220 to 240 mg/mL,

about 230 to 280 mg/mL, about 230 to 270 mg/mL, about 230 to 260 mg/mL, about 230 to 250 mg/mL, about 230 to 240 mg/mL, about 240 to 280 mg/mL, about 240 to 270 mg/mL, about 240 to 260 mg/mL, about 240 to 250 mg/mL, about 250 to 280 mg/mL, about 250 to 270 mg/mL, about 250 to 260 mg/mL, about 260 to 280 mg/mL, about 260 to 270 mg/mL, or about 270 to 280 mg/mL.

[00104] The inventions are further described by the following experiments and examples, which do not limit the inventions in any manner. The order of performance of the below experiments and/or examples or example steps can be altered or combined as determined by the person of skill in the art in view of the teachings and data contained herein.

EXAMPLES

[00105] Polysorbate 80 and polysorbate 20 were used since they are widely used throughout the industry. Although they both have POE sorbitan as their polar head, experiments were carried out to utilize both because polysorbates are highly heterogeneous, so even though the PS80 and PS20 heads were intended to be the same molecule, there may be differences in the concentration of POES or sPOES in each polysorbate solution. To manually produce the hydrolyzed PS for the experiments, the polysorbates' ester bond was broken by mixing the polysorbates with 0.1M sodium hydroxide and then incubated them at 45°C as described in Dwivedi, et. al. 2020 (Acidic and alkaline hydrolysis of polysorbates under aqueous conditions: Towards understanding polysorbate degradation in biopharmaceutical formulations. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences, 144, 105211).

[00106] In addition, three different monoclonal antibodies: mAb1 (dupilumab), mAb2 (targeting Bet v 1), and mAb3 (targeting Bet v 1) formulations were used. The mAb1 (dupilumab) formulation was selected because of its availability and the traces of hydrolytic proteins left in the solution. The mAb1 (dupilumab) and mAb2 (targeting Bet v 1) formulations were chosen because of their relatively high concentration of hydrolytic enzymes. Additionally, mAb3 (targeting Bet v 1), which didn't have a hydrolytic enzyme contamination, was selected as an example of mAb to confirm that the results are reproducible with multiple mAbs. Combined with the mAb solutions were their respective

buffers excluding the polysorbate so that the type of surfactant and the concentration of each surfactant in each sample during formulation could be manipulated.

[00107] Several methods were used to measure protein aggregation. Protein aggregation was primarily measured through high molecular weight percentage (HWM%) using size exclusion chromatography with high performance liquid chromatography (SEC-HPLC) and optical density at 405 nm (OD405 or turbidity). SEC and OD405 were the primary methods of measurement because they accounted for both soluble and insoluble protein aggregates. SEC was able to account for soluble protein aggregates, but was not able detect the insoluble aggregates. OD405 was used to account for the insoluble aggregates. This was because insoluble aggregates cause light scattering resulting in increased absorbance at all wavelengths. For stability studies the number of insoluble particles in solution also were measured using micro-flow imaging (MFI) at some of the time points. This was used as an additional measure of insoluble protein aggregates.

[00108] The measurements taken throughout the experiment include pH and the number of remaining PS in each sample using charged aerosol detector (CAD) to further narrow down the cause of the results to only one answer: the POES' effects.

[00109] To confirm that any protective effects recorded were caused by the polysorbate heads and not the hydrophilic (nonpolar) tails, the same trials were also ran with oleic acid (PS80 fatty acid ester) as the excipient. Polysorbates are highly heterogeneous solutions, so the experiments confirmed oleic acid and lauric acid as the main fatty acids released by the hydrolysis of PS80 and 20, respectively, as oleic acid takes up more than 90% of fatty acid esters in the polysorbate solution, while lauric acid takes up 40%-60%, as disclosed in Wuchner, et. al. 2022.

[00110] For the stability study, the samples were placed under 5°C conditions, the standard for protein drugs, as well as 25°C and 40°C to emulate stress conditions.

[00111] Experiments were carried out to evaluate the effectiveness of hydrolyzed polysorbates (PS) and the isolated POE sorbitan as excipients to replace traditional PS80 and PS20. This was achieved by using an agitation study to determine the short-term effectiveness, and an accelerated stability study to determine the long-term effectiveness of the hydrolyzed PS and POES or sPOES.

Materials:

[00112] Materials used for the experiments include 10% stock solution of Super-Refined polysorbate 80 (Tween® 80) and polysorbate 20 (Tween® 20) (from Croda (Princeton, NJ)). All other buffer ingredients (i.e., Sodium acetate, L-histidine, L- arginine-hydrochloride, sucrose) were multi-compendial grade. The buffer for mAb1 (dupilumab): for an initial concentration of 150 mg/mL was used in 12.5 mM Sodium Acetate, 20 mM L-Histidine, 25 mM L-Arginine Hydrochloride, 5% Sucrose (w/v), at pH 5.9 comprised of 20mM sodium acetate, 80mM histidine, 25mM arginine-hydrochloride, and 5% sucrose. The buffer for mAb2 was solely made of 10mM histidine and 5% sucrose. Sodium oleate was used as the salt to form oleic acid. The buffer for mAb2 (targeting Bet v 1): for an initial concentration of 150 mg/ml was used in 10 mM L-Histidine, 50 mM L-Arginine Hydrochloride, 5% Sucrose (w/v), at pH 6.0. The buffer for mAb3 (targeting Bet v 1): for an initial concentration of 150 mg/ml was used in 10 mM L-Histidine, 50 mM L-Arginine Hydrochloride, 5% Sucrose (w/v), at pH 6.0.

[00113] In these experiments, the mAbs (mAb1 (dupilumab), mAb2 (targeting Bet v 1), and mAb3 (targeting Bet v 1)) had initial concentrations of 150mg/mL and 50 mg/mL, used to represent subcutaneous and intravenous injection, respectively. Formulations were developed in glass vials and sealed with butyl rubber caps.

Hydrolysis and Purification of Polysorbates:

[00114] The polysorbates (PSs) were hydrolyzed and purified into POES. First, a 2% (w/v) PS solution was made. The PS solution was then hydrolyzed using 0.1M sodium hydroxide and incubated at 45°C for 24 hours (Dwivedi *et al.*, 2020). To purify the polysorbate, ethyl acetate was combined and mixed with the aqueous solution of the hydrolyzed PS. The ethyl acetate would only dissolve in the fatty acids, while the POES remained in water. The cloudy mixture was then poured into a separating funnel to drain out the fatty acids and the ethyl acetate, leaving only POES. This process was repeated up to ten times or until the POES layer was completely clear. **Figure 2** schematically illustrates hydrolysis of polysorbates and purification of POES. **Figure 2** also demonstrates the translation of the nominal value of POES% to the actual %.

[00115] **Figure 3** depicts hydrolyzed structure of polysorbate 80 (PS80), polyoxyethylene sorbitan (POES).

[00116] When the final POES solution was analyzed by Ultra Performance Liquid Chromatography with Charged Aerosol Detector (UPLC-CAD), it proved that the solution was almost completely free of polysorbates. In **Figures 4A and 4B**, the PS20 and PS80 lines illustrate normal polysorbate behavior, while the POES lines illustrate undegraded POES behavior. The smaller peaks at the beginning of each graph represent the POE, POE sorbitan, and the POE isosorbide that is present in both polysorbates and the POES solution. Although, the POE sorbitan concentration exceeds the concentrations of the other two components. The highest peaks in the PS20 and PS80 graphs are various intact polysorbates (Li, *et.al.* 2022. The measurement and control of high-risk host cell proteins for polysorbate degradation in biologics formulation. *Antibody therapeutics,* 5(1), 42–54). While the POES lines exhibit many of these peaks, the POES line stays almost completely flat, meaning there was little to no intact polysorbates left in the POES solution prepared for the experiments.

[00117] Referring back to **Figure 2**, the "2%" POES solution is the nominal value which is representative of the polysorbate concentration before the FFAs were removed, since the percentage is calculated by mass. The FFAs were removed so the solution had less solute per solvent, and because the percentage was calculated by mass, the actual POES concentration was lower than what is shown. For example, nominal 0.2% POES translates to an actual value of about 0.16% POES.

Formulations and Experiments:

[00118] Sample formulations involve the buffer (such as 5-20 mM Histidine, pH 5-7, 10-70 mM Arginine, 5-10% sucrose, and 5-20 mM Acetate), the polysorbate (such as 0.01-0.2% PS80) and mAbs at various concentrations. Prior to preparing the formulation, the protein (mAbs) and buffer were combined with the appropriate ratio to achieve 150 mg/mL or 50 mg/mL and then filtered (0.22-micron PVDF cup filter) to remove any impurities. During formulation, varying amounts of water and polysorbate were added to 6R vials to get the correct concentration of surfactant in each one. The polysorbate concentrations for mAbs were 0.005%, 0.01%, 0.025%, 0.05%, 0.1%, and 0.2% (**Table 1**). The same amount of mAb was added to each vial to finish the formulation. In total, 13 vials were prepared: 6 vials with different concentrations of regular polysorbate as controls, 6 vials with different concentrations of hydrolyzed PS or the isolated POES, and one vial with no surfactant to act as the 0% surfactant for the POES, hydrolyzed PS, and regular PS (**Table 1**). The vials were

capped and crimped before being agitated. Agitation was conducted in a vortex mixer (GENIE® SI-A236 Digital Vortex-Genie 2 Mixer, 120V) at 1000 rpm for two hours at 25ºC.

[00119] After agitation, the samples were measured using SEC-HPLC (Size Exclusion Chromatography 1200 Series LC Stacks, Water's Empower 2 software, HPLC columns) to obtain the High Molecular Weight percentage (HWM%) in order to measure soluble protein aggregates. To track insoluble aggregates, the optical density (OD) of each sample was recorded, where they were pipetted into a 96 well plate and then read by the OD machine (Molecular Devices SPECTRAmax 190 spectrophotometer with SoftMax Pro 6.2.1 software) at a wavelength of 405 nm. The remaining polysorbate concentration of each sample was quantified using CAD (Thermo (Dionex) Ultra RS charged aerosol detector, Waters Oasis MAX columns).

Table 1.

	PS/hPS/POES (%)	**Protein (mg/mL)**
A	0	150 or 50
B	0.005	150 or 50
C	0.01	150 or 50
D	0.025	150 or 50
E	0.05	150 or 50
F	0.1	150 or 50
G	0.2	150 or 50

Stability Study:

[00120] For the accelerated stability study, samples of regular polysorbate and POES containing the same concentrations of all ingredients were placed under 5°C, 25°C, and 40°C incubators. Furthermore, only POES from polysorbate 80 were used, since it is more widely used in the pharmaceutical industry and its protective abilities were more prominent than polysorbate 20 as confirmed by an initial agitation study. The study is for two years with time points set in between (2 weeks, 1 month, 2 months, 1 year, *etc.*).

mAb1 (dupilumab) Results:

[00121] After the samples were agitated in a vortex at 200 rpm for two hours at 25℃, data were collected for the HMW% and compared to the HMW% before agitation. The data were then graphed with the polysorbate or POES concentration as the x-axis and change in HMW% (HMW% after agitation v. HMW% before) as the y-axis to visualize how much protein was aggregated during agitation for each polysorbate concentration. **Figures 5-14** showing results using mAb1 (dupilumab) at a concentration of 150mg/mL.

[00122] **Figure 5** illustrates the change in high molecular weight percentage (HMW%) vs. the excipient concentrations for hydrolyzed polysorbate 80 compared to regular PS80 using mAb1 (dupilumab) at a concentration of 150mg/ml. **Figure 5** also shows the trend for regular PS80 is normal: as the concentration of PS80 increases, the amount of damaged protein decreases. The same trend is true for the hydrolyzed PS80. For both the hydrolyzed and non-hydrolyzed PS80, there was no HMW increase beyond 0.1% polysorbate. Therefore, the hydrolyzed PS80 is as effective as non-hydrolyzed PS80 at protecting mAb1 (dupilumab) from agitation.

[00123] Even though the graph generally followed an inverse relationship, but one point did not: the point with 0% surfactant. Although this would mean that there is little to no protein aggregation in the sample, when analyzed the OD data and visually inspected the samples, it was found that they had a very high OD increase and a turbidity that was not present before agitation, meaning that insoluble particles formed. This result aligned with our SEC data, since SEC cannot detect insoluble particles; it can only detect soluble particles. After analyzing the SEC data with the OD data, it is concluded that the protein aggregates within the 0% excipient sample were insoluble, meaning that SEC could not detect them.

[00124] While **Figure 5** displayed the effectiveness of the hydrolyzed PS (hPS) comprised of both the polyoxyethylene sorbitan and the oleic acid, the objective of the experiment was to see the effectiveness of only the POE sorbitan in protecting the proteins. In **Figure 6** is a graph showing the relationship between polysorbates 80 (non-hydrolyzed PS80 and isolated POES) and the change in HMW% in the samples using mAb1 (dupilumab) at a concentration of 150 mg/ml. **Figure 6** represents the relationship between the isolated POES with the change in HMW% before and after agitation. The regular PS80 produced the standard trend again: when the concentration of excipient increases, the HMW% decreases. There was also no HMW% increase after excipient concentrations of 0.05% for both the POES and regular PS80. This time, the POES was slightly more effective than the hydrolyzed

PS80, demonstrating the effectiveness of the POES alone at protecting mAbs from aggregation. **Figure 7** is showing the relationship between polysorbates 80 (non-hydrolyzed PS80 and isolated POES) and the change in ΔOD of the samples using mAb1 (dupilumab) at a concentration of 150mg/ml. It appears that the isolated POES is effective in protecting protein from agitation stress. Significant OD increase observed for 0% excipient only. It is evident from the graphs that the isolated POES is more effective in protecting proteins from agitation stress than hPS (POES + free fatty acids).

[00125] Testing was conducted to determine whether the protective effects on proteins are caused by the polysorbate residue or the hydrolyzed polysorbates. **Figure 8**, charged aerosol detector (CAD) data, graph showing that there are negligible traces of PS80 and PS20 left after hydrolysis (hydrolyzed PS-80). **Figure 8** graphs also showing that there are no traces of PS80 and PS20 left when PS20-POES or PS80-POES were used. Therefore, the data showing that the protective effects of the hydrolyzed polysorbates and POES (derived from PS20 or PS80) are not caused by the polysorbate residue. Additionally, the protective effects of oleic acid (PS80 free fatty acid) were tested to observe its potential protective effects on the mAb1 (dupilumab) alone. An experiment was conducted using oleic acid acting as the excipient. The data shown in **Figure 9** illustrates the relationship between the concentration of oleic acid and the change in HMW% after agitation (shaking) for two hours. It appears from **Figure 9** that sodium oleate (polysorbate 80 tails) as an excipient does not protect the proteins during agitation and makes protein aggregation worse. Unlike the graphs in **Figure 5** and **Figure 6**, as the concentration of excipient increases, the HMW increases with it. Additionally, there was a significant OD increase as the concentration of oleic acid increases. That OD increase was observed in the samples of oleic acid alone as well and is likely due to insoluble particles of the oleic acid itself.

[00126] **Figure 10** showing the relationship between polysorbates 20 (non-hydrolyzed PS20 and isolated POES) and the change in ΔOD of the samples using mAb1 (dupilumab) at a concentration of 150mg/ml. Therefore, oleic acid cannot act as a surfactant to protect against protein aggregation, showing that only the POES has the protective effect.

[00127] **Figure 11** is a graph showing the relationship between hydrolyzed PS20 and change in HMW concentration compared to regular PS20 in the samples using mAb1 (dupilumab) at a concentration of 150mg/ml. Data in this graph display that hydrolyzed PS20 is able to protect mAb1 (dupilumab) from aggregation. The graph in **Figure**

11, like the other graphs, shows the relationship between the concentration of hydrolyzed PS20 in mAb1 (dupilumab), and the change in HMW% after agitation. However, the hydrolyzed PS20 was less effective than the hydrolyzed PS80, since the change in HMW% was greater for the hydrolyzed PS20 than the regular PS20. The graph further illustrates an inverse relationship between the two factors for both the regular PS20 and the hydrolyzed PS20, revealing that like PS80, hydrolyzed PS20 also can work as a surfactant for proteins.

[00128] **Figure 12** is a graph showing the relationship between the concentration of POES to the change in concentration of HMW compared to regular PS20 in the samples using mAb1 (dupilumab) at a concentration of 150mg/ml. This graph also demonstrates, like the isolated POES from PS80 was more effective than the hydrolyzed PS80, the same trend was observed for the POES (heads) isolated from PS20. **Figure 12** is showing the relationship between POES from PS20 and the change in the HMW% after agitation for two hours compared to the regular PS20. Again, the effect of isolated POES on mAb1 (dupilumab) is very similar to that of PS20, as there is only a 0.1% difference in HMW increase between the POES and PS20 starting from excipient concentrations as little as 0.01%. These data demonstrate the POES isolated from PS20 is better than the hydrolyzed PS20 in preventing protein aggregation.

[00129] **Figure 13** is showing the relationship between polysorbates 80 (non-hydrolyzed PS80 and isolated POES) and the change in HMW% in the samples using mAb1 (dupilumab)at a concentration of 150mg/ml in an experiment that involved shaking protein solutions for 48 hours at 25°C.

[00130] **Figure 14** is showing the relationship between polysorbates 80 (non-hydrolyzed PS80 and isolated POES) and the change in ΔOD of the samples using mAb1 (dupilumab) at a concentration of 150mg/ml in an experiment that involved shaking (agitating) protein solutions for 48 hours at 25°C.

mAb2 (targeting Bet v 1) Results:

[00131] Along with mAb1 (dupilumab), the same experiments were also repeated with mAb2 (targeting Bet v 1). Data were plotted onto PS% vs. change in HMW% graphs and the results were the same: when there is an increase in excipient concentration, whether it be hydrolyzed or non-hydrolyzed, PS80 or PS20, there was always a significant decrease in protein aggregation. However, at excipient concentrations below 0.05%, the

ΔHMW% is abnormally high, so at low concentrations, POES from PS20 may not be as effective. Nevertheless, the HMW% in samples with POES did not increase more than that of the regular PS80 beyond excipient concentration levels of 0.05%. Overall, POES can protect mAb2 (targeting Bet v 1) as efficiently as mAb1 (dupilumab).

[00132] **Figure 15** is showing the relationship between polysorbates 80 (non-hydrolyzed PS80 and isolated POES) and the change in HMW% in the samples using mAb2 (targeting Bet v 1) at a concentration of 150mg/ml.

[00133] **Figure 16** is showing the relationship between polysorbates 80 (non-hydrolyzed PS80 and isolated POES) and the change in ΔOD of the samples using mAb2 (targeting Bet v 1) at a concentration of 150mg/ml.

[00134] **Figure 17** is a graph showing that isolated POES protects mAb2 (targeting Bet v 1) at a concentration of 50 mg/ml from agitation stress. The sample was subjected to 48 hours of rotation at 200 rpm at room temperature. The result shows that POES protects mAb from agitation stress.

mAb3 (targeting Bet v 1) Results:

[00135] **Figure 18** is a graph showing the relationship between non-hydrolyzed PS80 and isolated POES and the change in HMW% in the samples using mAb3 (targeting Bet v 1) at a concentration of 150 mg/ml.

[00136] **Figure 19** is showing the relationship between non-hydrolyzed PS80 and isolated POES and the change in ΔOD of the samples using mAb3 (targeting Bet v 1) at a concentration of 150 mg/ml.

[00137] **Figure 20** is showing the relationship between non-hydrolyzed PS20 and isolated POES and the change in HMW% in the samples using mAb3 (targeting Bet v 1) at a concentration of 150 mg/ml.

[00138] **Figure 21** illustrates the effect of agitation stress and the change in HMW% in the samples. Graph indicates that HMW% increase upon agitation stress. Data provide that hydrolyzed PS can effectively protect proteins from agitation stress.

Stability Studies of DP Formulations with Lipase Activity:

[00139] Polysorbates (PS20 and PS80) are used surfactants for mAb formulations. Monoclonal antibody formulations can be contaminated with lipases or esterases (from host cell proteins), which are difficult to remove during purification. Consequently, lipases and esterases often able to hydrolyze polysorbates thereby destabilizing drug products.

Preparation of Isolated POES:

[00140] Materials used for these experiments include 10% w/v solutions of PS20 and PS80, hydrolyzed with NaOH (at 45°C for 2 days) and neutralized with HCl (to pH about 6.0). POES was isolated from hydrolyzed polysorbates by extracting free fatty acids with ethyl acetate.

Synthetic POES (sPOES):

[00141] sPOES was synthesized by hydrolysis of PS20 and purified using HPLC method.

Agitation and Stability Studies:

[00142] Agitation studies were performed for mAbs with polysorbate as a control and following materials:

- 6R vial, 1 mL fill volume;
- Vortexing for 2 hours at, 1000 rpm; and
- Orbital rotation: 48h, 250 rpm, sideways.

Monoclonal Antibody (mAb) Formulations:

[00143] mAb1 (dupilumab): an initial concentration of 150 mg/mL was used in 12.5 mM Sodium Acetate, 20 mM L-Histidine, 25 mM L-Arginine Hydrochloride, 5% Sucrose (w/v), at pH 5.9.

[00144] mAb2 (targeting Bet v 1): an initial concentration of 150 mg/ml was used in 10 mM L-Histidine, 50 mM L-Arginine Hydrochloride, 5% Sucrose (w/v), at pH 6.0.

[00145] mAb3 (targeting Bet v 1): an initial concentration of 150 mg/ml was used in 10 mM L-Histidine, 50 mM L-Arginine Hydrochloride, 5% Sucrose (w/v), at pH 6.0.

[00146] Thermal stability study was performed for mAb1 (dupilumab) with high lipase activity using PS80 as a control (150 mg/mL mAb1 (dupilumab) in 12.5 mM

Sodium Acetate, 20 mM L-Histidine, 25 mM L-Arginine Hydrochloride, 5% Sucrose (w/v), 0.05% PS80 or POES, at pH 5.9). Samples were incubated in 6R glass vials (1 mL fill volume) with butyl rubber stoppers.

Results of Stability of mAb1 (dupilumab) in the Presence of Isolated POES:

[00147] **Figures 22A to 22C** illustrate the results of thermal stability studies of mAb1 (dupilumab) in the presence of isolated POES at 5ºC (**Fig. 22A**), 25ºC (**Fig. 22B**) and 40ºC (**Fig. 22C**). Results show that mAb1 (dupilumab) formulations with 0.05% POES and 0.05% PS-80 show comparable thermal stability.

Results of Surfactant Degradation in Lipase-Containing mAb1 (dupilumab) Formulations During Thermal Stability

[00148] **Figures 23A to 23C** illustrate the results of surfactant degradation studies in lipase-containing mAb1 (dupilumab) formulations during thermal stability at 5ºC (**Fig. 23A**), 25ºC (**Fig. 23B**) and 40ºC (**Fig. 23C**). No POES degradation was observed over time while PS80 degradation appeared significant under all thermal conditions tested.

[00149] **Figure 24** is a graph showing the subvisible particle (SVP) counts in lipase-containing mAb formulations during thermal stability studies at 40ºC at 75% relative humidity (RH). SVP counts were determined by using Micro-Flow Imaging (MFI). SVP count of POES mAb1 (dupilumab) formulation appeared to be much lower compared to PS80 mAb1 (dupilumab) formulation under the stress conditions studied.

Agitation Studies Using a Combination of POES and Trace PS80:

[00150] Agitation studies were performed for mAbs as described above. mAb1 (dupilumab) at a concentration of 150 mg/mL (formulation as described above) was used with 0.2% of either PS80 or POES.

[00151] **Figures 25A to 25D** showing the results of the agitation stress studies combining POES and PS80. It appeared that a combination of POES and trace amount of PS80 is synergistic and effectively protects mAb from agitation stress. **Figures 25A** (POES alone), **25B** (PS80 alone), **25C** (POES combined with 0.005% PS80), and **25D** (POES combined with 0.01% PS80) illustrate the change in HMW% vs. the surfactant PS80 and POES concentrations alone or in combinations.

[00152] **Figure 26** is a graph showing the results of synergistic effects of combination of POES and PS80 in protecting mAb formulations from agitation stress. The results show that low concentrations of POES (0.01% and above) were synergistically effective in protecting mAb1 (dupilumab) from agitation stress (orbital rotation, 48h) in combination with low concentrations of PS80 (0.005% or 0.01%) by reducing the HMW% aggregates.

[00153] **Figures 27A and 27B** also showing the results of the agitation stress studies and protection of mAb (dupilumab) formulations from agitation stress by combining POES and PS80. **Figures 27A** (PS80 alone) and **27B** (POES combined with no PS80, 0.005% PS80, or 0.01 % PS80) illustrate the change in turbidity (ΔOD405) vs. the surfactant concentrations. The results show that low concentrations of POES (0.01% and above) were synergistically effective in protecting mAb1 (dupilumab) from agitation stress (orbital rotation, 48h) in combination with low concentrations of PS80 (0.005% or 0.01%) by reducing turbidity ΔOD405 index.

Effect of sPOES on Protein Surface Binding:

[00154] Working Principles QCM-D monitoring is determined by: Δf (change in frequency) and ΔD (change in dissipation).

[00155] **Figure 28** schematically illustrates the process for evaluating the effect of synthetic POES (sPOES) on protein surface binding by Quartz Crystal Microbalance with Dissipation (QCM-D) monitoring.

Methods for Polyvinylidene Difluoride (PVDF) Binding Analysis by QCM-D:

[00156] Conditions and sequence for the analysis include: 50 µg/mL mAb1 (dupilumab), 20 mM histidine, at pH 6.0: (i) Buffer; (ii) Surfactant (if any); (iii) Protein + surfactant (if any); and (iv) Surfactant. Thickness of the protein layer was calculated from the QCM-D data. No binding of protein on the filter or container surface was preferred.

Synergistic Effect of Combination of POES and PS-80 on Protein-PVDF Interaction:

[00157] **Figure 29** is a bar graph showing the synergy and benefits of combining POES with PS80 on the absorption of mAb1 (dupilumab) to polyvinylidene difluoride (PVDF) membrane and protein-PVDF interaction (usually used as filter material). The results show that combination of 0.01% POES and and 0.01% PS80 were the most synergistic and effective in decreasing mAb1 (dupilumab) adsorption to PVDF surface than

PS80 or POES alone at these concentrations. Similar in effectiveness was also observed with 0.1% PS80. The results also provide that POES alone was not as effective as the combination of POES and PS80.

[00158] POES concentration was maintained during incubation with lipase-containing DP while PS-80 concentration dropped significantly. SVP count in isolated POES mAb1 (dupilumab) formulation was much lower compared to PS80 formulation under thermal stress conditions. Both isolated POES and sPOES showed comparable effects to PS80 in thermal stability of mAb1 (dupilumab). POES appeared to be less effective than PS80 in preventing protein binding to filters.

[00159] The inventions provide that polyoxyethylene sorbitan (POES) and/or sPOES released from hydrolysis of polysorbate can protect protein from agitation stress. The hydrolyzed polysorbate can still be effective in protecting proteins, including polypeptides, antibodies and mAbs from agitation stress. It is also provided that the POES and/or sPOES is effective in protecting proteins, including polypeptides, antibodies and mAbs from agitation stress, protecting the same from damage due to agitation and provides a stable formulation. Thus, POES or sPOES can be used as an excipient to replace commonly used surfactant in protein formulations.

[00160] Overall, both the hydrolyzed polysorbates (hPS80 and hPS20) and the isolated POES and/or sPOES are able to protect the mAbs from aggregation at high and low protein concentrations. However, the isolated POES and sPOES proved to be more effective than the hydrolyzed polysorbates. Furthermore, there are differences between the effectiveness of the PS80 and PS20, since the hPS80 and hPS20 are more effective than their counterparts based on the differences in their fatty acid esters. These differences make hPS80 a better choice as a surfactant to protect proteins from aggregation, because hPS20 is more likely to contribute to particle formation.

[00161] In general, the isolated POES of both the hPS80 and hPS20 were equally effective. Therefore, POES is a superior surfactant and excipient to replace polysorbates in the protein formulations, including therapeutic and pharmaceutical formulations.

[00162] Further, combination of POES and/or sPOES and PS (such as PS80 or PS20) have a synergistic effect and the combined effect is greater than the sum of their

separate effects. Combination of POES and PS in the formulations appeared to be more effective than either sPOES or PS80 individually in protecting mAbs from agitation stress. POES individually appeared to be less effective than PS80 individually in mitigating surface absorption of mAbs as determined by QCM-D. Combination of POES with 0.01% PS80 appeared to be effective in mitigating surface absorption of mAbs as determined by QCM-D.

[00163] It is to be understood that the description, specific examples and data, while indicating exemplary teachings, are given by way of illustration and are not intended to limit the present inventions. Various changes and modifications within the present inventions, including combining aspects in whole and in part, will become apparent to the skilled artisan from the discussion, disclosure and data contained herein, and thus are considered part of the inventions.

WHAT IS CLAIMED IS

1. A method of producing a stabilized protein formulation, wherein the method comprises the steps of

a. providing a protein solution, wherein the concentration of protein is about 5 to 300 mg/ml; and

b. adding polyoxyethylene sorbitan (POES) to the solution to a concentration of about 0.01 to 0.30% (w/v) to provide a stabilized formulation.

2. The method according to claim 1, wherein the wherein the stabilized formulation exhibits less than a 0.5% increase in aggregation after agitation as measured by SE-UPLC.

3. The method according to claim 1, wherein the wherein the stabilized formulation exhibits less than a 0.05 AU increase insoluble aggregates after agitation as measured by a spectrophotometer at OD405 nm.

4. The method according to claim 2 or 3, wherein the agitation is carried out by shaking the protein formulation in an upright orientation for about 60 to 120 minutes at about 1000 rpm and in a sideways orientation for about 24 to 48 hours at about 250 rpm at room temperature.

5. The method according to claim 1, wherein the concentration of protein is about 10 to 200 mg/ml.

6. The method according to claim 1, wherein the concentration of protein is about 50 to 150 mg/ml.

7. The method according to claim 1, wherein the concentration of protein is about 50 mg/ml.

8. The method according to claim 1, wherein the concentration of protein is about 150 mg/ml.

9. The method according to claim 1, wherein the concentration of the POES is about 0.01 to 0.25% (w/v), about 0.05 to 0.10% (w/v), about 0.10 to 0.15% (w/v), about 0.15 to 0.20% (w/v), about 0.20 to 0.25% (w/v), or about 0.25 to 0.30% (w/v).

10. The method according to claim 1, wherein the concentration of the POES is about 0.05% (w/v), about 0.10% (w/v), about 0.15% (w/v), about 0.20% (w/v), about 0.25% (w/v), or about 0.30% (w/v).

11. The method according to claim 1, wherein the protein is selected from the group consisting antibodies, antibody fragments or antibody derivatives.

12. The method according to claim 1, wherein the protein is an Fc-containing protein.

13. The method according to claim 12, wherein the Fc-containing protein is a receptor-Fc-fusion protein.

14. The method according to claim 13, wherein the receptor-Fc-fusion protein is a Trap protein.

15. The method according to claim 1, wherein the protein is an antibody.

16. The method according to claim 15, wherein the antibody is a monoclonal antibody.

17. The method according to claim 15, wherein the antibody is an IgG antibody.

18. The method according to claim 1, wherein the protein formulation is a liquid formulation.

19. The method according to claim 18, wherein the liquid formulation comprises pharmaceutically acceptable excipients including one or more buffer, salt and sugar.

20. A method of producing a stabilized protein formulation, wherein the method comprises the steps of

a. providing a protein solution, wherein the concentration of protein is about 5 to 300 mg/ml; and

b. adding hydrolyzed polysorbate (hPS) to the solution to a concentration of about 0.01 to 0.30% (w/v) to provide a stabilized formulation.

21. The method according to claim 20, wherein the wherein the stabilized formulation exhibits less than a 0.5% increase in aggregation after agitation as measured by SE-UPLC.

22. The method according to claim 20, wherein the wherein the stabilized formulation exhibits less than a 0.05 AU increase insoluble aggregates after agitation as measured by a spectrophotometer at OD405 nm.

23. The method according to claim 21 or 22, wherein the agitation is carried out by shaking the protein formulation in an upright orientation for about 60 to 120 minutes at about 1000 rpm and in a sideways orientation for about 24 to 48 hours at about 250 rpm at room temperature.

24. The method according to claim 20, wherein the concentration of protein is about 10 to 200 mg/ml.

25. The method according to claim 20, wherein the concentration of protein is about 50 to 150 mg/ml.

26. The method according to claim 20, wherein the concentration of protein is about 50 mg/ml.

27. The method according to claim 20, wherein the concentration of protein is about 150 mg/ml.

28. The method according to claim 20, wherein the hPS is hydrolyzed polysorbate 80 (hPS80) or hydrolyzed polysorbate 20 (hPS20).

29. The method according to claim 20, wherein the concentration of the hPS is about 0.01 to 0.25% (w/v), about 0.05 to 0.10% (w/v), about 0.10 to 0.15% (w/v), about 0.15 to 0.20% (w/v), about 0.20 to 0.25% (w/v), or about 0.25 to 0.30% (w/v).

30. The method according to claim 20, wherein the concentration of the hPS is about 0.05% (w/v), about 0.10% (w/v), about 0.15% (w/v), about 0.20% (w/v), about 0.25% (w/v), or about 0.30% (w/v).

31. The method according to claim 20, wherein the protein is selected from the group consisting antibodies, antibody fragments or antibody derivatives.

32. The method according to claim 20, wherein the protein is an Fc-containing protein.

33. The method according to claim 32, wherein the Fc-containing protein is a receptor-Fc-fusion protein.

34. The method according to claim 33, wherein the receptor-Fc-fusion protein is a Trap protein.

35. The method according to claim 20, wherein the protein is an antibody.

36. The method according to claim 35, wherein the antibody is a monoclonal antibody.

37. The method according to claim 35, wherein the antibody is an IgG antibody.

38. The method according to claim 20, wherein the protein formulation is a liquid formulation.

39. The method according to claim 38, wherein the liquid formulation comprises pharmaceutically acceptable excipients including one or more buffer, salt and sugar.

40. A stabilized protein formulation produced by a method comprising the steps of

a. providing a protein solution, wherein the concentration of protein is about 5 to 300 mg/ml; and

b. adding hydrolyzed polysorbate (hPS) or isolated polyoxyethylene sorbitan (POES) to the solution to a concentration of about 0.01 to 0.30% (w/v) to provide a stabilized formulation.

41. The stabilized protein formulation of claim 40, wherein the formulation is resistant to agitation.

42. The stabilized protein formulation of claim 40, wherein the stabilized formulation exhibits less than a 0.5% increase in aggregation after agitation as measured by SE-UPLC.

43. The stabilized protein formulation of claim 40, wherein the wherein the stabilized formulation exhibits less than a 0.05 AU increase insoluble aggregates after agitation as measured by a spectrophotometer at OD405 nm.

44. The stabilized protein formulation of claim 41, 42, or 43, wherein the agitation is carried out by shaking the protein formulation in an upright orientation for about 60 to 120 minutes at about 1000 rpm and in a sideways orientation for about 24 to 48 hours at about 250 rpm at room temperature.

45. The stabilized protein formulation of claim 1, wherein the concentration of protein is about 10 to 200 mg/ml.

46. The stabilized protein formulation of claim 40, wherein the concentration of protein is about 50 to 150 mg/ml.

47. The stabilized protein formulation of claim 40, wherein concentration of protein is about 50 mg/ml.

48. The stabilized protein formulation of claim 40, wherein the concentration of protein is about 150 mg/ml.

49. The stabilized protein formulation of claim 40, wherein the hPS is hydrolyzed polysorbate 80 (hPS80) or hydrolyzed polysorbate 20 (hPS20).

50. The stabilized protein formulation of claim 40, wherein the concentration of the hPS or isolated POES is about 0.01 to 0.25% (w/v), about 0.05 to 0.10% (w/v), about 0.10 to 0.15% (w/v), about 0.15 to 0.20% (w/v), about 0.20 to 0.25% (w/v), or about 0.25 to 0.30% (w/v).

51. The stabilized protein formulation of claim 40, wherein the concentration of the hPS or isolated POES is about 0.05% (w/v), about 0.10% (w/v), about 0.15% (w/v), about 0.20% (w/v), about 0.25% (w/v), or about 0.30% (w/v).

52. The stabilized protein formulation of claim 40, wherein the protein is selected from the group consisting antibodies, antibody fragments or antibody derivatives.

53. The stabilized protein formulation of claim 40, wherein the protein is an Fc-containing protein.

54. The stabilized protein formulation of claim 53, wherein the Fc-containing protein is a receptor-Fc-fusion protein.

55. The stabilized protein formulation of claim 54, wherein the receptor-Fc-fusion protein is a Trap protein.

56. The stabilized protein formulation of claim 40, wherein the protein is an antibody.

57. The stabilized protein formulation of claim 56, wherein the antibody is a monoclonal antibody.

58. The stabilized protein formulation of claim 56, wherein the antibody is an IgG antibody.

59. The stabilized protein formulation of claim 40, wherein the protein formulation is a liquid formulation.

60. The stabilized protein formulation of claim 40, wherein the liquid formulation comprises pharmaceutically acceptable excipients including one or more buffer, salt and sugar.

61. A method of producing a stabilized protein formulation, wherein the method comprises the steps of

a. providing a protein solution, wherein the concentration of protein is about 5 to 300 mg/ml; and

b. adding polyoxyethylene sorbitan (POES) to the solution to a concentration of about 0.01 to 0.30% (w/v) and about 0.001% (w/v) to about 0.30% (w/v) of PS80 or PS20 to provide a stabilized formulation.

62. The method according to claim 61, wherein the wherein the stabilized formulation exhibits less than a 0.5% increase in aggregation after agitation as measured by SE-UPLC.

63. The method according to claim 61, wherein the wherein the stabilized formulation exhibits less than a 0.05 AU increase insoluble aggregates after agitation as measured by a spectrophotometer at OD405 nm.

64. The method according to claim 62 or 63, wherein the agitation is carried out by shaking the protein formulation in an upright orientation for about 60 to 120 minutes at about 1000 rpm and in a sideways orientation for about 24 to 48 hours at about 250 rpm at room temperature.

65. The method according to claim 61, wherein the concentration of protein is about 10 to 200 mg/ml.

66. The method according to claim 61, wherein the concentration of protein is about 50 to 150 mg/ml.

67. The method according to claim 61, wherein the concentration of protein is about 50 mg/ml.

68. The method according to claim 61, wherein the concentration of protein is about 150 mg/ml.

69. The method according to claim 61, wherein the concentration of the POES, PS80 or PS20 is about 0.01 to 0.25% (w/v), about 0.05 to 0.10% (w/v), about 0.10 to 0.15% (w/v), about 0.15 to 0.20% (w/v), about 0.20 to 0.25% (w/v), or about 0.25 to 0.30% (w/v).

70. The method according to claim 61, wherein the concentration of the POES, PS80 or PS20 is about 0.05% (w/v), about 0.10% (w/v), about 0.15% (w/v), about 0.20% (w/v), about 0.25% (w/v), or about 0.30% (w/v).

71. The method according to claim 61, wherein the concentration of the POES, PS80 or PS20 is about 0.01% (w/v).

72. The method according to claim 61, wherein the concentration of the POES is about 0.01% (w/v) and the concentration of PS80 or PS 20 is about 0.005% (w/v).

73. The method according to claim 61, wherein the protein is selected from the group consisting antibodies, antibody fragments or antibody derivatives.

74. The method according to claim 61, wherein the protein is an Fc-containing protein.

75. The method according to claim 74, wherein the Fc-containing protein is a receptor-Fc-fusion protein.

76. The method according to claim 75, wherein the receptor-Fc-fusion protein is a Trap protein.

77. The method according to claim 61, wherein the protein is an antibody.

78. The method according to claim 77, wherein the antibody is a monoclonal antibody.

79. The method according to claim 77, wherein the antibody is an IgG antibody.

80. The method according to claim 61, wherein the protein formulation is a liquid formulation.

81. The method according to claim 80, wherein the liquid formulation comprises pharmaceutically acceptable excipients including one or more buffer, salt and sugar.

82. A stabilized protein formulation produced by a method comprising the steps of

a. providing a protein solution, wherein the concentration of protein is about 5 to 300 mg/ml; and

b. adding hydrolyzed polysorbate (hPS) or isolated polyoxyethylene sorbitan (POES) to the solution to a concentration of about 0.01 to 0.30% (w/v) and about 0.01% (w/v) to about 0.30% (w/v) of PS80 or PS20 to provide a stabilized formulation.

83. The stabilized protein formulation of claim 82, wherein the formulation is resistant to agitation.

84. The stabilized protein formulation of claim 82, wherein the stabilized formulation exhibits less than a 0.5% increase in aggregation after agitation as measured by SE-UPLC.

85. The stabilized protein formulation of claim 82, wherein the wherein the stabilized formulation exhibits less than a 0.05 AU increase insoluble aggregates after agitation as measured by a spectrophotometer at OD405 nm.

86. The stabilized protein formulation of claim 83, 84, or 85, wherein the agitation is carried out by shaking the protein formulation in an upright orientation for about 60 to 120 minutes at about 1000 rpm and in a sideways orientation for about 24 to 48 hours at about 250 rpm at room temperature.

87. The stabilized protein formulation of claim 82, wherein the concentration of protein is about 10 to 200 mg/ml.

88. The stabilized protein formulation of claim 82, wherein the concentration of protein is about 50 to 150 mg/ml.

89. The stabilized protein formulation of claim 82, wherein concentration of protein is about 50 mg/ml.

90. The stabilized protein formulation of claim 82, wherein the concentration of protein is about 150 mg/ml.

91. The stabilized protein formulation of claim 82, wherein the hPS is hydrolyzed polysorbate 80 (hPS80) or hydrolyzed polysorbate 20 (hPS20).

92. The stabilized protein formulation of claim 82, wherein the concentration of the hPS, isolated POES, PS80 or PS20 is about 0.01 to 0.25% (w/v), about 0.05 to 0.10% (w/v), about 0.10 to 0.15% (w/v), about 0.15 to 0.20% (w/v), about 0.20 to 0.25% (w/v), or about 0.25 to 0.30% (w/v).

93. The stabilized protein formulation of claim 82, wherein the concentration of the hPS, isolated POES, PS80 or PS20 is about 0.05% (w/v), about 0.10% (w/v), about 0.15% (w/v), about 0.20% (w/v), about 0.25% (w/v), or about 0.30% (w/v).

94. The stabilized protein formulation of claim 82, wherein the concentration of the hPS, POES, PS80 or PS20 is about 0.01% (w/v).

95. The stabilized protein formulation of claim 82, wherein the concentration of the hPS, POES is about 0.01% (w/v) and the concentration of PS80 or PS 20 is about 0.005% (w/v).

96. The stabilized protein formulation of claim 82, wherein the protein is selected from the group consisting antibodies, antibody fragments or antibody derivatives.

97 The stabilized protein formulation of claim 82, wherein the protein is an Fc-containing protein.

98. The stabilized protein formulation of claim 97, wherein the Fc-containing protein is a receptor-Fc-fusion protein.

99. The stabilized protein formulation of claim 98, wherein the receptor-Fc-fusion protein is a Trap protein.

100. The stabilized protein formulation of claim 82, wherein the protein is an antibody.

101. The stabilized protein formulation of claim 100, wherein the antibody is a monoclonal antibody.

102. The stabilized protein formulation of claim 100, wherein the antibody is an IgG antibody.

103. The stabilized protein formulation of claim 82, wherein the protein formulation is a liquid formulation.

104. The stabilized protein formulation of claim 82, wherein the liquid formulation comprises pharmaceutically acceptable excipients including one or more buffer, salt and sugar.