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(54) Title: STABLE PROTEIN FORMULATIONS

(57) Abstract: The invention provides a protein formulation comprising a polyol, of enhanced stability. The invention further provides methods of stabilizing a protein formulation by determining the Reverse Stability Concentration for a protein in a formulation comprising a polyol. The invention additionally provides methods of stabilizing protein formulations by reducing the amount of polyol in the composition. The invention provides antibody formulations having long-term stability and advantageous characteristics for subcutaneous administration.

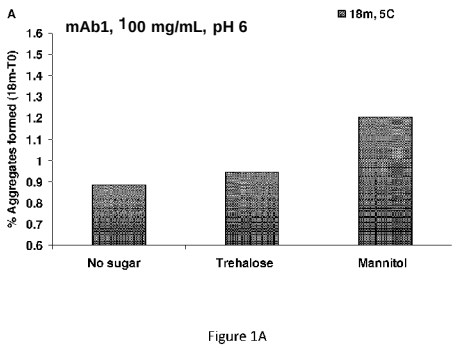

Figure 1A

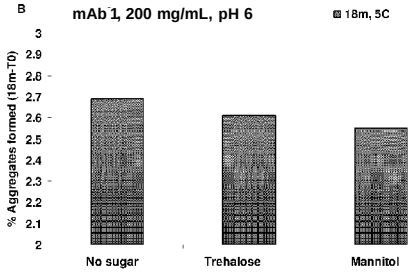

Figure 1B

MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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STABLE PROTEIN FORMULATIONS

RELATED APPLICATIONS

This application claims priority to United States Provisional Application No. 61/580,046, filed on December 23, 2011, the entire contents of which are incorporated herein by reference.

BACKGROUND

The formulation of therapeutic proteins, such as antibodies, is often a challenge given the numerous desirable properties that the formulation must have to be economically and therapeutically successful, *e.g.,* stability, suitability for administration, concentration, and manufacturability. During manufacturing, storage, and delivery, therapeutic proteins have been known to undergo physical and chemical degradations. These instabilities can reduce the potency of the protein and increase the risk of adverse events in patients, and, therefore, significantly impact regulatory approval (see, *e.g.,* Wang, *et al.* (2007) J Pharm Sci 96:1). As such, a stable protein formulation is essential to the safety and efficacy of a successful therapeutic protein drug product.

To be effective, many therapeutic proteins require the administration of particular doses, which, preferably, are formulated at specific concentrations. High protein concentration formulations are desirable as they can impact the mode *{e.g.,* intravenous vs. subcutaneous) and frequency of administration of the drug to a subject (eg., daily vs. weekly). Furthermore, access to high concentrations can reduce the injection volume and thus foster feasibility of user-friendly delivery devices such as pens, autoinjectors, or micro-needle mediated transdermal delivery devices, and thus increase treatment adherence and patient convenience.

Many sugars *{e.g.,* sucrose, glucose, raffinose, trehalose) and polyols *{e.g.,* glycerol, sorbitol, mannitol) are subsumed under the category of protein stabilizing co-solvents. It is generally accepted that these substances act primarily through a preferential exclusion mechanism and hence stabilize by conformational stabilization. Many research groups have verified experimentally that stabilizers such as polyols are preferentially excluded from the protein surface, leading to an increase of the protein's chemical potential. Since the unfolded state has a larger surface area, the extent of exclusion is greater for the unfolded state, resulting in a higher increase of the chemical potential of that unfolded form. Consequently,

the free energy difference between native and unfolded/denatured forms is increased, resulting in an increased structural intrinsic stability of the native protein. (Arakawa, T. and Timasheff, S.N. Mechanism of poly(ethylene glycol) interaction with proteins. Biochemistry, 24:6756-6762 (1985); Carpenter, J.F. and Crowe, J.H. Mechanism of stabilization of proteins by solutes. Cryobiology, 25:244 255 (1988); Arakawa, T., Kita, Y. and Carpenter, J.F. Protein-solvent interactions in pharmaceutical formulations. Pharm. Res., 8:285-291 (1991); Bhat, R. and Timasheff, S.N. Steric exclusion is the principal source of the preferential hydration of proteins in the presence of polyethylene glycols. Protein Science, 1:1133-1143 (1992); Timasheff, S.N. A physicochemical basis for the selection of osmolytes by nature. In G. Somero *et al.* (eds.), Water and Life, Springer Verlag, Berlin, 1992, pp. 70 84). Researchers also found that the amount of sugars and polyols present in protein formulations directly correlates with intrinsic protein stability, *e.g.,* the extent of protein stability in liquid protein formulations containing sucrose, raffinose, trehalose, or glucose positively correlates with sugar/polyol concentration, meaning the less sugar present in the formulation, the higher the extent of aggregation found (Foster *et al,* Int. J. Pharm.(1996) 134(1,2): 193-201).

This current state of the art has been reviewed by Manning *et al.* (Pharm. Res. 27(4):544-575 (2010)), stating that "the ability of low-molecular- weight additives to increase the free energy of unfolding was well known in 1989, based on many publications by Timasheff and coworkers" *{see, e.g.,* Lee JC and Timasheff SN. J Biol Chem 256:7193-7201 (1982); Arakawa T. and Timasheff S.N. Biochemistry 21:6536-6544 (1982); Timasheff SN. Adv Protein Chem 51:355-432 (1998); Timasheff SN. Methods Mol Biol 40:253-269 (1995); and Arakawa T. and Timasheff S.N. Biophys J. 47:41 1-414 (1985)). Manning *et al.* further state that "the application of this approach as a general formulation strategy was only beginning to emerge at that time. Since then, numerous examples of conformational stabilization by excluded solutes have been reported. We now know that osmolytes operate by this general mechanism to provide increased conformational stability to proteins. Thus, nearly any sugar or polyol should increase the structural stability of a protein. In addition, it has also been shown that most amino acids act as excluded solutes ... as do a number of salts ... and many polymers, including gelatin ... and even poloxamers " *{see,* for example, Kita Y *et al.* Biochemistry 33:15178-15189 (1994); Gheibi N. *et al.* J Biosci 31:355-362 (2006); Kar K. *et al.* J Chem thermodyn 34:319-336 (2002); Vrkljan M. *et al.* Pharm Res 11:1004-1008 (1994); and Stratton LP *et al.* J Pharm Sci 86:1006-1012 (1997)). Consistent with the current understanding articulated by Manning *et al.,* polyols such as sorbitol are often used to

stabilize parenterals such as, for example, a number of lyophilized vaccine pharmaceuticals such as Mumpsvax™, Meruvax™ II and Attenuvax™ or intravenous administrable solutions such as Cardene™. Thus, it is not surprising that the overwhelming majority of monoclonal antibody and Ig-fusion protein formulations currently on the market or for which market approval has been applied with regulatory agencies also contain sugars and/or polyols to stabilize the protein, especially for high protein concentrations, such as Cimzia, Enbrel, Remicade, Xolair, Herceptin, Stelara, Simponi and Ozespa.

Stability of protein formulations, in particular the susceptibility to aggregation, is primarily determined by the conformational and the colloidal stability of the protein molecules. It is generally believed that the first step in non-native protein aggregation, which is the most prevalent form of aggregation, is a slight perturbation of the molecular structure, *e.g.,* a partial unfolding of the protein, *i.e.,* a conformational change. This is determined by the conformational stability of the protein. In the second step, the partially unfolded molecules then come into close proximity, being driven by diffusion and random Brownian motion, to form aggregates. This second step is primarily governed by the colloidal stability of the molecules (see Chi *et al.*, Roles of conformational stability and colloidal stability in the aggregation of recombinant human granulocyte colony stimulating factor. Protein Science, 2003 May; 12(5): 903-913). It is generally understood that sugars provide stability to protein formulations by enhancing the conformational or the structural stability of the molecules, *i.e.* by impacting the first step in aggregation.

Differential scanning calorimetry (DSC) and differential scanning fluorimetry (DSF) are well known techniques in the art that are used to predict the stability of a protein formulation. Specifically, these techniques can be used to determine the unfolding temperature (Tm) of a protein in given formulation. It is standard in the art to correlate high Tm measurements for a protein in given formulation with more robust and stable protein formulations for long-term, shelf-stable storage. Polyols, by raising the Tm of protein formulations as determined by DSC and DSF, are considered to be beneficial for enhancing the stability of the formulations during long-term, shelf stable storage conditions (see Burton *et al.,* Use of Microcalorimetry and Its Correlation with Size Exclusion Chromatography for Rapid Screening of the Physical Stability of Large Pharmaceutical Proteins in Solution, Pharm Dev Technol, 2007; 12(3) 265-273).

SUMMARY OF THE INVENTION

The present invention is based, at least in part, on the surprising discovery that at particular protein concentrations, polyols are destabilizing to protein formulations. Specifically, Applicants have discovered that for protein formulations, a Reserve Stability Concentration (RSC) of the protein exists, wherein below the RSC, the presence of polyols is detrimental to the long-term stability of the formulation. In contrast, when the concentration of the protein in the formulation is above the RSC, the presence of polyols is beneficial for the long-term stability of the formulations. Accordingly, the present invention provides a means to develop protein formulations with maximal stability, thus maintaining functionality of the pharmaceutical protein over the desired time frame and reducing patient risk and efficacy failures due to delivering suboptimal protein formulations.

In one aspect, the invention provides a protein formulation having enhanced shelf-life stability relative to a first protein formulation that comprises a polyol at a first polyol concentration, wherein the protein formulation comprises a protein at a concentration below a Reverse Stability Concentration for the protein in the first protein formulation; and no polyol or a polyol at a concentration below the first polyol concentration. In one embodiment, the protein concentration in the protein formulation is greater than the protein concentration in the first protein formulation. In some embodiments, the formulations are low ionic formulations, *e.g.,* comprise a buffer, such as a phosphate buffer, a citrate buffer, a citrate and phosphate buffer, a histidine buffer, an acetate buffer, or a succinate buffer, and do not comprise additional ionic excipient(s), such as NaCl. In some embodiments, the formulation is substantially free of protein aggregation in a liquid form at 5°C for a period of time selected from the group consisting of at least 3 months, at least 6 months, at least 12 months, at least 18 months and at least 24 months. Aspects of the invention feature formulations with protein aggregation at a level less than 10% protein aggregation, less than 5% protein aggregation, less than 2% protein aggregation or less than 1% aggregation following storage at room temperature for at least 3 months. In some embodiments, the polyol concentration is below about 200 mg/ml, below about 120 mg/ml, below about 80 mg/ml or below about 40 mg/ml. In one embodiment, the Reverse Stability Concentration of the protein is between about 100 mg/mL and 150 mg/mL. In another embodiment, the Reverse Stability Concentration of the protein is between about 125 mg/mL and 135 mg/mL. In some embodiments, the protein concentration is at least about 25 mg/mL, at least about 50 mg/ml, at least about 100 mg/ml or at least about 150 mg.

Additional aspects of the invention include protein formulations having one or more distinct proteins, wherein at least one of the proteins is a therapeutic protein. In one embodiment, the protein is an antibody, or an antigen-binding portion thereof. In one embodiment, the antibody, or an antigen-binding portion thereof, comprises a lambda light chain or a kappa light chain. In one embodiment, the protein formulation contains a polyol that is sucrose, trehalose, mannitol and/or sorbitol.

Additional aspects of the invention include methods of treating a disorder in a subject by administering to the subject a protein formulation of the invention.

Further aspects of the invention include methods of determining the Reverse Stability Concentration of a protein in a formulation comprising a polyol. In one embodiment, a method of determining the Reverse Stability Concentration of a protein includes preparing a series of formulations comprising a protein and a polyol, wherein the protein concentration in the formulations is constant and the polyol concentration is varied over a range of concentrations, and wherein the concentration of other solutes in the formulations is the same, exposing the series of formulations to a test condition, measuring the level of protein aggregation in the series of formulations after exposure to the test condition; and determining the presence of a change in the level of aggregation in the series of formulations over the range of polyol concentration, wherein an absence of a change in the level of aggregation in the series of formulations over the range of polyol concentrations indicates that the protein concentration is the Reverse Stability Concentration of the protein in the formulation.

In one embodiment, a method of detecting the Reverse Stability Concentration of a protein in a formulation comprising a polyol includes preparing a first series of formulations comprising a protein at a first protein concentration and a polyol, wherein the polyol concentration is varied over a range, preparing a second series of formulations comprising a protein at a second protein concentration that is higher than the first protein concentration and a polyol, wherein the polyol concentration is varied over a range, and wherein the concentration of other solutes in the first series of formulations and second series of formulations is the same, exposing the first series of formulations and the second series of formulations to a test condition, measuring the level of protein aggregation in the first series of formulations and the second series of formulations after exposure to the test condition; and determining the change in the level of aggregation in each series of formulations from lowest to highest polyol concentration, wherein an increase in the level of aggregation in the first series of formulations and a decrease in the level of aggregation in the second series indicates

that the Reverse Stability Concentration is between the first protein concentration and the second protein concentration for the protein in a protein formulation with a polyol.

In one embodiment, a method of detecting the existence of a Reverse Stability Concentration of a protein in a formulation comprising a polyol at a given ionic strength includes preparing a first formulation and a second formulation, wherein the concentration of polyol is lower in the first formulation than in the second formulation, and wherein the concentration of the protein in the first formulation and second formulation is the same, exposing the first and second formulations to a test condition; and measuring protein aggregation in the first and second formulations after testing, wherein greater protein aggregation in the second formulation as compared to the first formulation indicates that a Reverse Stability Concentration exists for the protein in the formulation comprising the polyol.

In some embodiments, the formulations are low ionic formulations, *e.g.,* comprise a buffer, such as a phosphate buffer, a citrate buffer, a citrate and phosphate buffer, a histidine buffer, an acetate buffer, or a succinate buffer, and do not comprise additional ionic excipient(s), such as NaCl. In some embodiments, the test condition comprises storage at a temperature of 5°C, 40°C or 50°C. In one embodiment, the test condition comprises storage for a period of time of at least about 3 months, at least 6 months, at least 12 months, at least 18 months or at least 24 months. In one embodiment, protein aggregation is measured by size exclusion HPLC, UPLC, or nephelometry. In some embodiments, the protein is a therapeutic protein. In some embodiments, the protein is an antibody, or an antigen-binding portion thereof. In some embodiments, the antibody, or an antigen-binding portion thereof, comprises a lambda light chain or a kappa light chain.

Aspects of the invention include formulations where the protein concentration is below about 125 mg/ml. Aspects of the invention include formulations where the protein concentration is below about 100 mg/ml. In one embodiment, the protein concentration is below about 80 mg/ml. In one embodiment, the protein concentration is below about 60 mg/ml. In one embodiment, the protein concentration is below about 40 mg/ml. In one embodiment, the polyol is trehalose, mannitol, sucrose and/or sorbitol. In some embodiments, the formulations are low ionic formulations, *e.g.,* comprise a buffer and do not comprise additional ionic excipient(s), such as NaCl.

Embodiments of the invention include methods for reducing protein aggregation in a formulation comprising a protein and a polyol, comprising determining a Reverse Stability

Concentration for the protein in the formulation, and reducing the concentration of the polyol in the formulation if the protein concentration is lower than the Reverse Stability Concentration, thereby reducing protein aggregation in the formulation.

In one embodiment, a method for reducing protein aggregation in a formulation comprising a protein and a polyol comprises selecting a formulation comprising a protein and a polyol, identifying the protein concentration in the formulation as above or below the Reverse Stability Concentration for the protein in the formulation; and reducing the concentration of the polyol when the protein concentration is below the Reverse Stability Concentration for the protein in the formulation, thereby reducing protein aggregation in the formulation.

In one embodiment, a method for preparing a stable formulation having increased protein concentration as compared to a first formulation comprises selecting a first formulation comprising a protein at a first protein concentration and a polyol at a first polyol concentration, determining a Reverse Stability Concentration for the protein in the first formulation; increasing the protein concentration in the formulation to a concentration that is above the first protein concentration and below the Reverse Stability Concentration; and reducing the concentration of the polyol in the formulation below the first polyol concentration, thereby preparing a stable formulation having increased protein concentration as compared to the first formulation. In some embodiments, the formulations are low ionic formulations, *e.g.,* comprise a buffer, such as a phosphate buffer, a citrate buffer, a citrate and phosphate buffer, a histidine buffer, an acetate buffer, or a succinate buffer, and do not comprise additional ionic excipient(s), such as NaCl. In some embodiments, the polyol concentration is reduced to less than about 200 mg/ml. In some embodiments, the polyol concentration is reduced to less than about 120 mg/ml. In some embodiments, the polyol concentration is reduced to less than about 80 mg/ml. In some embodiments, the polyol concentration is reduced to less than about 40 mg/ml. In some embodiments, the polyol concentration is reduced to the polyol concentration is reduced to 0.

In some embodiments, the protein formulation is substantially free of protein aggregation in a liquid form at 5°C for a period of time of at least 3 months, at least 6 months, at least 12 months, at least 18 months or at least 24 months. In one embodiment, the protein formulation comprises protein aggregation at a level of less than 10% protein aggregation following storage at room temperature for at least 3 months. In one embodiment, the protein formulation comprises protein aggregation at a level of less than 5% protein aggregation

following storage at room temperature for at least 3 months. In one embodiment, the protein formulation comprises protein aggregation at a level of less than 2% protein aggregation following storage at room temperature for at least 3 months. In one embodiment, the protein formulation comprises protein aggregation at a level of less than 1% aggregation following storage at room temperature for at least 3 months.

Additional aspects of the invention include methods for reducing protein aggregation in a formulation comprising a protein and a polyol, comprising the steps of selecting a formulation comprising a protein and a polyol, identifying the protein concentration in the formulation as above the Reverse Stability Concentration for the protein in the formulation; and increasing the concentration of the polyol, thereby reducing protein aggregation in the formulation. One embodiment of the invention includes methods for preparing a stable formulation having increased protein concentration as compared to a first formulation, the method comprising: selecting a first formulation comprising a protein at a first protein concentration and a polyol at a first polyol concentration, determining a Reverse Stability Concentration for the protein in the first formulation, increasing the protein concentration in the formulation to a concentration that is above the first protein concentration and above the Reverse Stability Concentration; and increasing the concentration of the polyol in the formulation above the first polyol concentration, thereby preparing a stable formulation having increased protein concentration as compared to the first formulation.

The present invention is further illustrated by the following detailed description and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and B show the impact of sugar (80 mg/ml trehalose and 40 mg/ml mannitol) on the aggregation of ABT-736 at 100 mg/ml and 200 mg/ml, respectively, following 18m at 5°C.

Figure 2A shows the impact of sucrose concentration (% sucrose) on aggregation at 40°C of ABT-736 formulations of different protein concentrations following 3 m storage. Note that the 1 mg/mL data has been taken from samples stored at 50°C for 14 days. Also note that the 1 mg/ml sample data show negative results for aggregates because the time 0 samples had more aggregates than time X samples.

Figure 2B shows the impact of sucrose concentration (% sucrose) on aggregation at 40°C of ABT-874 formulations of different protein concentrations following 3 m storage. Note that the 1 mg/mL data has been taken from samples stored at 50°C for 14 days.

Figures 3A and B show data for ABT-736 and ABT-874, respectively, in terms of protein concentration (X axis) vs. slope (% aggregation vs. % sucrose, over varying sucrose concentrations at a fixed protein concentration, as depicted in Figures 2A and B). Low ionic strength data is from Figures 2A and B while high ionic strength data was obtained from samples stored at 50°C for 9 days.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides protein formulations, *e.g.,* antibody formulations which are substantially free of protein aggregation and which are, thus, more stable and easier to administer to subjects. The invention further provides methods of preparing stable protein formulations. The present invention is based, at least in part, on the surprising discovery that polyols typically used in protein formulations to stabilize protein may act as effective destabilizers. It was unexpectedly found that below a certain concentration of the protein *(i.e.,* at protein concentrations below the Reverse Stability Concentration, or RSC), sugars and polyols increase aggregation *(e.g.,* in solutions of low ionic strength, *e.g.,* solutions comprising a buffer but no additional ionic excipient(s)). Above this concentration of reverse stability, however, polyols do stabilize the protein. Thus, reducing the amount of sugar in protein formulations, *e.g.,* formulations comprising protein at a concentration below the RSC, results in an increase in protein stability. In contrast, at protein concentrations above the RSC, sugars and polyols decrease aggregation *(e.g.,* in solutions of low ionic strengths, such as below 30 mM).

I. Definitions

In order that the present invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention.

The term "pharmaceutical formulation" and/or "pharmaceutical composition" refers to preparations which are in such form as to permit the biological activity of the active

ingredients to be unequivocally effective, and which contain no additional components which are significantly toxic to the subjects to which the formulation would be administered.

The phrase "pharmaceutically acceptable carrier" is art recognized and includes a pharmaceutically acceptable material, composition or vehicle, suitable for administration to mammals. The carriers include liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject agent from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to or impacting safety of the patient.

"Pharmaceutically acceptable excipients" (vehicles, additives, buffers, etc.) are those which can reasonably be administered to a subject mammal to provide an effective dose of the active ingredient employed or to maintain stability of the pharmaceutical composition.

The term "pharmaceutical", as used herein, refers to a composition, *e.g.,* an aqueous formulation, that it is useful for treating a disease or disorder.

The term "subject" is intended to include living organisms, *e.g.,* prokaryotes and eukaryotes. Examples of subjects include mammals, *e.g.,* humans, dogs, cows, horses, pigs, sheep, goats, cats, mice, rabbits, rats, and transgenic non-human animals. In specific embodiments of the invention, the subject is a human.

The term "excipient" refers to an agent which may be added to a formulation to provide a desired consistency, *e.g.,* altering the bulk properties, to improve stability, and/or to adjust osmolality. Examples of commonly used excipients include, but are not limited to, sugars, polyols, amino acids, surfactants, and polymers.

A commonly used excipient is a polyol. As used herein, a "polyol" is a substance with multiple hydroxyl groups, and includes sugars (reducing and nonreducing sugars), sugar alcohols and sugar acids. Preferred polyols herein have a molecular weight which is less than about 600 kD. Non-limiting examples of polyols are fructose, mannose, maltose, lactose, arabinose, xylose, ribose, rhamnose, galactose, glucose, sucrose, trehalose, sorbose, melezitose, raffinose, mannitol, xylitol, erythritol, threitol, sorbitol, glycerol, L-gluconate and metallic salts thereof. A "reducing sugar" is one that contains a hemiacetal group that can reduce metal ions or react covalently with lysine and other amino groups in proteins and a "nonreducing sugar" is one that does not have these properties of a reducing sugar. Examples of reducing sugars are fructose, mannose, maltose, lactose, arabinose, xylose, ribose, rhamnose, galactose and glucose. Nonreducing sugars include sucrose, trehalose, sorbose,

melezitose and raffinose. Mannitol, xylitol, erythritol, threitol, sorbitol and glycerol are examples of sugar alcohols. As to sugar acids, these include L-gluconate and metallic salts thereof. The polyol may also act as a tonicity agent.

The term "ionic excipient" or "ionizable excipient," as used interchangeably herein, refers to an agent that has a net charge. In one embodiment, the ionic excipient has a net charge under certain formulation conditions, such as pH. Examples of an ionic excipient include, but are not limited to, histidine, arginine, and sodium chloride. The term "non-ionic excipient" or "non-ionizable excipient," as used interchangeably herein, refers to an agent having no net charge. In one embodiment, the non-ionic excipient has no net charge under certain formulation conditions, such as pH. Examples of non-ionic excipients include, but are not limited to, sugars *(e.g.,* sucrose), sugar alcohols *(e.g.,* mannitol), and non-ionic surfactants *(e.g.,* polysorbate 80).The term "lyoprotectant" as used herein includes agents that provide stability to a protein during water removal during the drying or lyophilisation process, for example, by maintaining the proper conformation of the protein. Examples of lyoprotectants include saccharides, in particular di- or trisaccharides. Cryoprotectants may also provide lyoprotectant effects.

The term "protein" is meant to include a sequence of amino acids for which the chain length is sufficient to produce the higher levels of secondary and/or tertiary and/or quaternary structure. The term protein is intended to include peptides that do not have such structure. In one embodiment, the proteins used herein have a molecular weight of at least about 1 kD, 5 kD, 10 kD, 20 kD, 30 kD, 40 kD or more. Examples of proteins encompassed within the definition used herein include therapeutic proteins. A "therapeutically active protein" or "therapeutic protein" refers to a protein which may be used for therapeutic purposes, *i.e.,* for the treatment of a disorder in a subject. It should be noted that while therapeutic proteins may be used for treatment purposes, the invention is not limited to such use, as said proteins may also be used for *in vitro* studies. In a preferred embodiment, the therapeutic protein is a fusion protein or an antibody, or antigen-binding portion thereof. In one embodiment, the methods and compositions of the invention comprise at least two distinct proteins, which are defined as two proteins having distinct amino acid sequences. Additional distinct proteins do not include degradation products of a protein.

As used herein, "buffer" refers to a buffered solution that resists changes in pH by the action of its acid-base conjugate components. The buffers of this invention have a pH ranging from about 4 to about 8; preferably from about 4.5 to about 7; and most preferably has a pH in

the range from about 5.0 to about 6.5. However, the surprising findings of this invention are the consequence of protein-polyol interactions or the interaction of effects exerted on the protein by the presence of a polyol in the composition, *e.g.,* effects on activity, osmolarity, density, etc. Thus, the invention applies to protein formulations for any pH value at which the protein is formulated. Examples of buffers that will control the pH in this range include, *e.g.,* phosphate, acetate *(e.g.,* sodium acetate), succinate *(e.g.,* sodium succinate), gluconate, glutamate, histidine, citrate and other organic acid buffers.

The term "surfactant" generally includes those agents which protect a protein in a formulation from air/solution interface-induced stresses and solution/surface induced- stresses. For example, a surfactant may protect the protein from aggregation. Suitable surfactants may include, *e.g.,* polysorbates, polyoxyethylene alkyl ethers such as Brij 35.RTM, or poloxamer such as Tween 20, Tween 80, or poloxamer 188. Preferred detergents are poloxamers, *e.g.,* Poloxamer 188, Poloxamer 407; polyoxyethylene alkyl ethers, *e.g.*,Brij 35.RTM., Cremophor A25, Sympatens ALM/230; and polysorbates/T weens, *e.g.,* Polysorbate 20, Polysorbate 80, Mirj, and Poloxamers, *e.g.,* Poloxamer 188, and Tweens, *e.g.,* Tween 20 and Tween 80.

The term "stabilizing agent" refers to an excipient that improves or otherwise enhances stability. Stabilizing agents include, but are not limited to, a-lipoic acid, a-tocopherol, ascorbyl palmitate, benzyl alcohol, biotin, bisulfites, boron, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ascorbic acid and its esters, carotenoids, calcium citrate, acetyl-L-camitine, chelating agents, chondroitin, chromium, citric acid, coenzyme Q-10, cysteine, cysteine hydrochloride, 3-dehydroshikimic acid (DHS), EDTA (ethylenediaminetetraacetic acid; edetate disodium), ferrous sulfate, folic acid, fumaric acid, alkyl gallates, garlic, glucosamine, grape seed extract, gugul, magnesium, malic acid, metabisulfite, N-acetyl cysteine, niacin, nicotinomide, nettle root, ornithine, propyl gallate, pycnogenol, saw palmetto, selenium, sodium bisulfite, sodium metabisulfite, sodium sulfite, potassium sulfite, tartaric acid, thiosulfates, thioglycerol, thiosorbitol, tocopherol and their esters, *e.g.,* tocopheral acetate, tocopherol succinate, tocotrienal, d-a—tocopherol acetate, vitamin A and its esters, vitamin B and its esters, vitamin C and its esters, vitamin D and its esters, vitamin E and its esters, *e.g.,* vitamin E acetate, zinc, and combinations thereof.

As used herein, the term "tonicity modifier" is intended to mean a compound or compounds that can be used to adjust the tonicity of a liquid formulation. Suitable tonicity modifiers include glycerin, lactose, mannitol, dextrose, sodium chloride, magnesium sulfate, magnesium chloride, sodium sulfate, sorbitol, trehalose, sucrose, raffinose, maltose and others

known to those or ordinary skill in the art. In one embodiment, the tonicity of the liquid formulation approximates that of the tonicity of blood or plasma. As used herein, the term "acidic component" refers to an agent, including a solution, having an acidic pH, *i.e.,* less than 7.0. Examples of acidic components include phosphoric acid, hydrochloric acid, acetic acid, citric acid, oxalic acid, succinic acid, tartaric acid, lactic acid, malic acid, glycolic acid and fumaric acid. In one embodiment, the aqueous formulation of the invention does not include an acidic component.

As used herein, the term "basic component" refers to an agent which is alkaline, *i.e.,* pH greater than 7.0. Examples of basic components include potassium hydroxide (KOH) and sodium hydroxide (NaOH)As used herein, the term "antioxidant" is intended to mean an agent which inhibits oxidation and thus is used to prevent the deterioration of preparations by the oxidative process. Such compounds include by way of example and without limitation, acetone, sodium bisulfate, ascorbic acid, ascorbyl palmitate, citric acid, butylated hydroxyanisole, butylated hydroxytoluene, hydrophosphorous acid, monothioglycerol, propyl gallate, methionine, sodium ascorbate, sodium citrate, sodium sulfide, sodium sulfite, sodium bisulfite, sodium formaldehyde sulfoxylate, thioglycolic acid, sodium metabisulfite, EDTA (edetate), pentetate and others known to those of ordinary skill in the art.

As used herein, the term "bulking agent" is intended to mean a compound used to add bulk to the reconstitutable solid and/or assist in the control of the properties of the formulation during preparation. Such compounds include, by way of example and without limitation, dextran, trehalose, sucrose, polyvinylpyrrolidone, lactose, inositol, sorbitol, dimethylsulfoxide, glycerol, albumin, calcium lactobionate, and others known to those of ordinary skill in the art.

The term "cryoprotectants" as used herein generally includes agents, which provide stability to the protein from freezing-induced stresses. Examples of cryoprotectants include polyols such as, for example, mannitol, and include saccharides such as, for example, sucrose, as well as including surfactants such as, for example, polysorbate, poloxamer or polyethylene glycol, and the like. Cryoprotectants also contribute to the tonicity of the formulations.

A "stable" composition or formulation is one in which the protein, *e.g.,* antibody, therein essentially retains its physical stability and/or chemical stability and/or biological activity during the manufacturing process and/or upon storage. Various analytical techniques for measuring protein stability are available in the art and are reviewed in Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs.

(1991) and Jones, A. (1993) Adv. Drug Delivery Rev. 10: 29-90. For example, in one embodiment, the stability of the protein is determined according to the percentage of monomer protein in the solution, with a low percentage of degraded *(e.g.,* fragmented) and/or aggregated protein. Preferably, the formulation is stable at room temperature (about 30 °C) or at 40 °C for at least 1 month and/or stable at about 2-8° C for at least 6 months, or for at least 1 year or for at least 2 years. Furthermore, the formulation is preferably stable following freezing (to, *e.g.,* -70 °C) and thawing of the formulation, hereinafter referred to as a "freeze/thaw cycle."

A protein, *e.g.,* an antibody, "retains its physical stability" in a pharmaceutical formulation if it shows substantially no signs of instability, *e.g.,* aggregation, precipitation and/or denaturation, upon visual examination of color and/or clarity or as measured by UV light scattering or by size exclusion chromatography. Aggregation is a process whereby individual protein molecules or complexes associate covalently or non-covalently to form aggregates. Aggregation can proceed to the extent that a visible precipitate is formed.

"Substantial protein aggregation" refers to a level of protein aggregation in a protein formulation that is substantially greater than the level of protein aggregation in a first *(e.g.,* reference) protein formulation. The first protein formulation may be the same protein formulation before a period of storage or before a treatment *(e.g.,* the first protein formulation may be a formulation of protein of time zero (TO) when the formulation is initially prepared or of time zero before the formulation is subjected to a destabilizing condition, such as elevated temperature, and/or to long term storage.).

The phrase "a composition which is substantially free of protein aggregation" refers to protein formulations of the invention that do not have a significantly greater level or percentage of aggregated protein than a first *(e.g.,* reference) formulation. For example, this phrase refers to compositions in which the level of protein aggregation is less than about 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.2% or 0.1%. In one embodiment, the level of protein aggregation is less than about 2%. The level of protein aggregation in a composition may be determined using standard techniques known in the art, and as described in the Examples herein.

The phrase "concentration of polyol in the composition that does not result in substantial protein aggregation" refers to a concentration of polyol in the formulation that is sufficiently low at a temperature of, *e.g.,* 5 °C, 25 °C or 40 °C such that a normal or acceptable level of aggregation present in the composition is observed, *e.g.,* the aggregate

level observed in a corresponding normal stressed lot, *e.g.,* about 1.5% aggregation. For example, the concentration of polyol in the formulation is such that only less than about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, 2%, 3%, 4%, 5%, 8%, 10% of aggregation is observed. In one embodiment, the concentration of polyol in the formulation is such that only less than about 2% aggregation is observed.

Stability, such as physical stability of a composition or formulation, may be assessed by methods well-known in the art, including measurement of a sample's apparent attenuation of light (absorbance, or optical density). Such a measurement of light attenuation relates to the turbidity of a formulation. The turbidity of a formulation is partially an intrinsic property of the protein dissolved in solution and is commonly determined by nephelometry, and measured in Nephelometric Turbidity Units (NTU).

The degree of turbidity, *e.g.,* as a function of the concentration of one or more of the components in the solution, *e.g.,* protein and/or salt concentration, is also referred to as the "opalescence" or "opalescent appearance" of a formulation. The degree of turbidity can be calculated by reference to a standard curve generated using suspensions of known turbidity. Reference standards for determining the degree of turbidity for pharmaceutical compositions can be based on the European Pharmacopeia criteria (European Pharmacopoeia, Fourth Ed., Directorate for the Quality of Medicine of the Council of Europe (EDQM), Strasbourg, France). According to the European Pharmacopeia criteria, a clear solution is defined as one with a turbidity less than or equal to a reference suspension which has a turbidity of approximately 3 according to European Pharmacopeia standards. Nephelometric turbidity measurements can detect Rayleigh scatter, which typically changes linearly with concentration, in the absence of association or nonideality effects. Other methods for assessing physical stability of a pharmaceutical protein are well-known in the art, *e.g.,* size-exclusion chromatography or analytical ultracentrifucation.

A protein, *e.g.,* antibody, "retains its chemical stability" in a pharmaceutical formulation, if the chemical stability at a given time is such that the protein, *e.g.,* antibody, is considered to still retain its biological activity *(e.g.,* as defined below for antibody). Chemical stability can be assessed by, *e.g.,* detecting and quantifying chemically altered forms of the protein, *e.g.,* antibody. Chemical alteration may involve size modification *(e.g.* clipping) which can be evaluated using size exclusion chromatography, SDS-PAGE and/or matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI/TOF MS), for

example. Other types of chemical alteration include charge alteration *(e.g.* occurring as a result of deamidation or oxidation) which can be evaluated by ion-exchange chromatography, for example.

A protein, *e.g.,* antibody, "retains its biological activity" in a pharmaceutical formulation, if the protein, *e.g.,* antibody, in a pharmaceutical formulation is biologically active for its intended purpose. For example, biological activity is retained if the biological activity of the protein, *e.g.,* antibody, in the pharmaceutical formulation is within about 30%, about 20%, or about 10% (within the errors of the assay) of the biological activity exhibited at the time the pharmaceutical formulation was prepared *(e.g.,* as determined in an antigen binding assay).

The stability of a protein formulation may be "improved" by removing or adding a component of the formulation, by changing the amount of the component in the formulation or the concentration of a component in the formulation. In one embodiment, an amount of a component *(e.g.,* polyol) of a first protein formulation is reduced while keeping the amount of all other components the same, thereby improving the stability of the protein formulation.

The term "shelf-life stability" refers to the stability of a formulation under storage conditions that are substantially similar to conditions under which the formulation would be stored as a commercial product for use. In one embodiment of the invention, "shelf-life stability" is distinguished from stability of a formulation when stored or tested under conditions that promote the instability, *i.e.* unfolding or aggregating, of the protein(s) in the formulation.

"Treatment" in the context of a protein formulation refers to a process done to the formulation or condition under which the formulation is placed in order to test the effects of the components of the formulation on the stability of the protein of the formulation.

As used herein, a "reverse stability concentration" of a protein or a "reverse stability protein concentration" is the concentration of the protein for any polyol concentration (polyol concentration at least in the pharmaceutically relevant range of 10-200 mg/ml) at which there is no net effect of any concentration of the the polyol (at least the concentration of the polyol of interest in pharmaceutical formulations, *e.g.,* < 120 mg/ml for disaccharides and < 80 mg/ml for monosacharides) on the stability of protein in the protein formulation. Addition of polyol at a protein concentration higher than this would be stabilizing while addition of polyol at a protein concentration lower than this would be destabilizing. In some embodiments of the invention, the presence of a reverse stability concentration for a protein will be dependent on

the ionic strength of the formulation (e.g., will exist in low ionic strength protein formulations). In some embodiments of the invention, increasing the ionic strength of a formulation will reduce the reverse stability concentration. In some embodiments of the invention, increasing the ionic strength of a formulation will eliminate the reverse stability concentration, *i.e.*, by increasing the ionic strength of a formulation, the addition of a polyol will be stabilizing regardless of protein concentration. In one embodiment, the stability of the protein is measured in terms of protein aggregation, *e.g.*, percentage of protein aggregation. Examples of a reverse stability concentration of a protein include, *e.g.*, 10 mg/ml, 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml, 100 mg/ml, 110 mg/ml, 120 mg/ml, 130 mg/ml, 140 mg/ml, 150 mg/ml, 160 mg/ml, 170 mg/ml, 180 mg/ml, 190 mg/ml or 200 mg/ml. In one embodiment, a reverse stability concentration of a protein is, *e.g.*, between 100-160 mg/ml, between 110-150 mg/ml, between 120-140 mg/ml or between 125-135 mg/ml. In one embodiment, a reverse stability concentration of a protein is about 130 mg/ml. In another embodiment, the reverse stability concentration of a protein in the protein formulation {*e.g.*, formulations of Stelara or Simponi or high concentration protein formulations that contain polyols) is the concenctration below which a decrease in the polyol concentration would lead to an increase in stability of protein in the protein formulation at both recommended storage temperature and temperature of administration {*e.g.*, 5°C, ambient, or 37°C).

"Substantially free of monomer loss" refers to a percentage of protein that is in monomer form in a protein formulation that is, *e.g.*, about 95%, 96%, 97%, 98%, 99% or greater.

"Substantially free of fragments" refers to a fragmented protein concentration in a protein formulation that is, *e.g.*, about 5.0 mg/ml, 4.0 mg/ml, 3.0 mg/ml 2.0 mg/ml, 1.0 mg/ml, 0.5 mg/ml, 0.4 mg/ml, 0.3 mg/ml, 0.2 mg/ml or less.

A "a protein concentration below the reverse stability concentration of a protein in the formulation" refers to a protein concentration in the formulations of the invention at which an increase in the polyol concentration of the formulation would reduce stability of the protein in the protein formulation. In one embodiment, a a protein concentration below the reverse stability concentration of a protein in the formulation is a protein concentration in the formulation at a concentration where a reduction in the polyol concentration of the formulation increases stability of the protein in the formulation. In some embodiments, a "a protein concentration below the reverse stability concentration of a protein in the formulation"

is, *e.g.,* at least about 50 mg/ml, 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml, 100 mg/ml, 120 mg/ml, 140 mg/ml, 160 mg/ml, or 180 mg/ml.

A "protein concentration above the reverse stability concentration of a protein in the formulation" refers to a protein concentration in the formulations of the invention at which an increase in the polyol concentration of the formulation would increase stability of the protein in the protein formulation. In some embodiments, a "protein concentration above the reverse stability concentration of a protein in the formulation" is, *e.g.,* at least 80 mg/ml, 90 mg/ml, 100 mg/ml, 120 mg/ml, 140 mg/ml, 160 mg/ml, 180 mg/ml, 200 mg/ml, 220 mg/ml, 240 mg/ml, 260 mg/ml, 280 mg/ml or 300 mg/ml. In some embodiments, a "protein concentration above the reverse stability concentration of a protein in the formulation" is, *e.g.,* at least about 120 mg/ml, 125 mg/ml, 130 mg/ml, 135 mg/ml, 140 mg/ml, or 150 mg/ml.

A "low ionic strength" or "low ionic" formulation refers to a formulation with an ionic strength such that a protein comprised by the formulation will have a reverse stability concentration, *e.g.,* at certain low protein concentrations, the presence of a polyol, *e.g.,* mannitol, sucrose, trehalose, sorbitol, in the formulation will not have a stabilizing effect and at higher protein concentrations, the presence of a polyol in the formulation will have a stabilizing effect. A low ionic formulation of the invention is a protein formulation comprising a buffer (such as a phosphate buffer, a citrate buffer, a citrate and phosphate buffer, a histidine buffer, an acetate buffer, or a succinate buffer) and that does not contain additional ionic excipients, *e.g.,* sodium chloride. As used herein, a "series of formulations" or "formulation series" refers to a set of two or more compositions comprising a protein, water, and other ingredients, wherein the concentration of ingredients in the compositions, other than the protein and any polyols, is fixed or constant. In one embodiment of the invention, a series of formulations comprises a "first formulation" and a "second formulation".

Aspects of the invention include improvements to protein formulations that enhance stability and shelf-life. Accordingly, improvements in protein stability can be seen by comparing a first protein formulation (also known herein as a "reference formulation") to a second protein formulation, which may differ in terms of composition *(e.g.,* presence, amount, or concentration of one or more components, or their chemical, molecular or ionic form(s)), treatment or age. First formulations can vary from second formulations based on one or more aspects of the formulations. In one embodiment, the first formulation contains one or more of the components of a second formulation at a different concentration as compared to the concentration of those components in the second formulation and one or more of the

components of the second formulation at the same concentration as compared to the concentration of those components in the second formulation. In one embodiment of the invention, the first formulation and the second formulation comprise a protein and a polyol, and further comprise additional components which are present at the same concentration in each formulation. In one embodiment of the invention, all of the components of the first and second formulations have the same concentration, except for the concentration of a protein or the concentration of a polyol. In another embodiment, the concentration of all components in the first and second formulations are the same, except for the concentration of a protein and a polyol. In another embodiment of the invention, all of the components of the first and second formulations are the same, but the treatment *(e.g.,* storage conditions, testing conditions) and/or the amount of time that has elapsed since the formulations were made *(i.e.,* the age of the formulations) is/are different.

A "first protein formulation" refers to a protein formulation comprising the same protein as a second protein formulation but differing in other components *(e.g.,* presence, amount, or concentration of one or more components, or their chemical, molecular or ionic form(s)), protein concentration, treatment, manufacture and/or age. In one embodiment of the invention, a first protein formulation is compared to a second protein formulation with the same formulation that has been stored under different storage conditions. In one embodiment of the invention, a first protein formulation is compared to a second protein formulation that has a different concentration of the protein. In one embodiment, a first protein formulation is compared to a second protein formulation that has a specific polyol concentration, known as the "second formulation polyol concentration", which may be the same, about the same or significantly different from the polyol concentration of the first protein formulation.

As used herein, "reducing the concentration of a polyol" refers to the reformulation of a composition to contain less polyol than a previous formulation of the composition. In one embodiment, "reducing the concentration of a polyol" refers to the reduction or removal of a polyol from a composition. In one embodiment, the reduction or removal of a polyol from a composition is performed with ultrafiltration or dialysis.

In a pharmacological sense, in the context of the present invention, a "therapeutically effective amount" or "effective amount" of an antibody refers to an amount effective in the prevention or treatment or alleviation of a symptom of a disorder for the treatment of which the antibody is effective. A "disorder" is any condition that would benefit from treatment with

the antibody. This includes chronic and acute disorders or diseases including those pathological conditions which predisposes the subject to the disorder in question.

The term "antibody" broadly refers to any immunoglobulin (Ig) molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, interconnected by disulfide bonds or any functional fragment, mutant, variant, or derivation thereof, which retains the essential epitope binding features of an Ig molecule. Such mutant, variant, or derivative antibody formats are known in the art, nonlimiting embodiments of which are discussed herein.

In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CHI, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of 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. Immunoglobulin molecules can be of any type *(e.g.,* IgG, IgE, IgM, IgD, IgA and IgY), class *(e.g.,* IgG 1, IgG2, IgG 3, IgG4, IgAl and IgA2) or subclass.

The term "Fc region" refers to the C-terminal region of an immunoglobulin heavy chain, which may be generated by papain digestion of an intact antibody. The Fc region may be a native sequence Fc region or a variant Fc region. The Fc region of an immunoglobulin generally comprises two constant domains, a CH2 domain and a CH3 domain, and optionally comprises a CH4 domain. Replacements of amino acid residues in the Fc portion to alter antibody effector function are known in the art (US Patent Nos: 5,648,260 and 5,624,821). The Fc portion of an antibody mediates several important effector functions, *e.g.,* cytokine induction, antibody dependent cell mediated cytotoxicity (ADCC), phagocytosis, complement dependent cytotoxicity (CDC) and half-life/ clearance rate of antibody and antigen-antibody complexes. Certain human IgG isotypes, particularly IgGl and IgG3, mediate ADCC and CDC via binding to FcyRs and complement Clq, respectively. The dimerization of two identical heavy chains of an immunoglobulin is mediated by the dimerization of CH3 domains and is stabilized by the disulfide bonds within the hinge region (Huber *et al.* (1976) Nature

264:415-20; Thies *et al.* (1999) J. Mol. Biol. 293:67-79). Mutation of cysteine residues within the hinge regions to prevent heavy chain-heavy chain disulfide bonds destabilizes dimeration of CH3 domains. Residues responsible for CH3 dimerization have been identified (Dall'Acqua (1998) Biochem. 37:9266-73). Therefore, it is possible to generate a monovalent half-Ig. Monovalent half Ig molecules have been found in nature for both IgG and IgA subclasses (Seligman (1978) Ann. Immunol. 129:855-70; Biewenga *et al.* (1983) Clin. Exp. Immunol. 51:395-400). A half Ig molecule may have certain advantages in tissue penetration due to its smaller size than that of a regular antibody. In one embodiment, at least one amino acid residue is replaced in the constant region of the binding protein of the invention, for example the Fc region, such that the dimerization of the heavy chains is disrupted, resulting in half Ig molecules. The light chain may be either a kappa or lambda type.

As used herein, the term "CDR" refers to the complementarity determining region within a antibody variable sequence. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Id.) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia *et al.* found that certain sub-portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence (Chothia *et al.* (1987) Mol. Biol. 196:901-917; Chothia *et al.* (1989) Nature 342:877-883) These sub-portions were designated as LI, L2 and L3 or HI, H2 and H3 where the "L" and the "H" designates the light chain and the heavy chains regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan (1995) FASEB J. 9:133-139 and MacCallum (1996) J. Mol. Biol. 262(5):732-45. Still other CDR boundary definitions may not strictly follow one of the herein described systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although certain embodiments use Kabat or Chothia defined CDRs.

The term "antigen-binding portion" of an antibody or "antibody portion" includes fragments of an antibody that retain the ability to specifically bind to an antigen *(e.g.*, hIL-12 and/or hIL-23). Such antibody embodiments may also be bispecific, dual specific, or multi-specific, *e.g.*, it specifically binds to two or more different antigens. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CHI domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CHI domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123). Such antibody binding portions are known in the art (Kontermann and Dubel eds. (2001) Antibody Engineering, Springer-Verlag, New York. pp. 790. In addition, single chain antibodies also include "linear antibodies" comprising a pair of tandem Fv segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions (Zapata *et al.* (1995) Protein Eng. 8(10): 1057-1062; US Patent No. 5,641,870).

Still further, an antibody or antigen-binding portion thereof may be part of a larger immunoadhesion molecules, formed by covalent or non-covalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such

immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, S.M., *et al.* (1995) *Human Antibodies and Hybridomas* 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov, S.M., *et al.* (1994) *Mol. Immunol.* 31:1047-1058). Antibody portions, such as Fab and $F(ab')_2$ fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein. Preferred antigen binding portions are complete domains or pairs of complete domains.

The term "bispecific antibody" refers to full-length antibodies that are generated by quadroma technology (Milstein, C. and A.C. Cuello (1983) Nature 305(5934):537-40), by chemical conjugation of two different monoclonal antibodies (Staerz, U.D. *et al.* (1985) Nature 314(6012):628-31), or by knob-into-hole or similar approaches that introduce mutations in the Fc region (Holliger, P. *et al.* (1993) Proc. Natl. Acad. Sci. USA 90:6444-8.18), resulting in multiple different immunoglobulin species of which only one is the functional bispecific antibody. By molecular function, a bispecific antibody binds one antigen (or epitope) on one of its two binding arms (one pair of HC/LC), and binds a different antigen (or epitope) on its second arm (a different pair of HC/LC). By this definition, a bispecific antibody has two distinct antigen binding arms (in both specificity and CDR sequences), and is monovalent for each antigen to which it binds.

The term "dual-specific antibody" refers to a full-length antibody that can bind two different antigens (or epitopes) in each of its two binding arms (a pair of HC/LC) (PCT Publication No. WO 02/02773). Accordingly, a dual-specific binding protein has two identical antigen binding arms, with identical specificity and identical CDR sequences, and is bivalent for each antigen to which it binds.

An immunoglobulin constant domain refers to a heavy or light chain constant domain. Human IgG heavy chain and light chain constant domain amino acid sequences are known in the art.

Two antibody domains are "complementary" where they belong to families of structures which form cognate pairs or groups or are derived from such families and retain this feature. For example, a VH domain and a VL domain of an antibody are complementary; two

VH domains are not complementary, and two VL domains are not complementary. Complementary domains may be found in other members of the immunoglobulin superfamily, such as the Vα and Vβ (or gamma and delta) domains of the T-cell receptor.

The term "domain" refers to a folded protein structure which retains its tertiary structure independently of the rest of the protein. Generally, domains are responsible for discrete functional properties of proteins, and in many cases may be added, removed or transferred to other proteins without loss of function of the remainder of the protein and/or of the domain. By single antibody variable domain is meant a folded polypeptide domain comprising sequences characteristic of antibody variable domains. It therefore includes complete antibody variable domains and modified variable domains, for example in which one or more loops have been replaced by sequences which are not characteristic of antibody variable domains, or antibody variable domains which have been truncated or comprise N- or C-terminal extensions, as well as folded fragments of variable domains which retain at least in part the binding activity and specificity of the full-length domain.

Variable domains of the invention may be combined to form a group of domains; for example, complementary domains may be combined, such as VL domains being combined with VH domains. Non-complementary domains may also be combined. Domains may be combined in a number of ways, involving linkage of the domains by covalent or non-covalent means.

A "dAb" or "domain antibody" refers to a single antibody variable domain (V_H or V_L) polypeptide that specifically binds antigen.

The term "monoclonal antibody" or "mAb" refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each mAb is directed against a single determinant on the antigen. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method. In an embodiment, the monoclonal antibody is produced by hybridoma technology.

The term "epitope" is meant to refer to that portion of any molecule capable of being recognized by and bound by an antibody at one or more of the antibody's antigen binding regions. In the context of the present invention, first and second "epitopes" are understood to

be epitopes which are not the same and are not bound by a single monospecific antibody, or antigen-binding portion thereof.

The phrase "recombinant antibody" refers to antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial antibody library, antibodies isolated from an animal *(e.g.,* a mouse) that is transgenic for human immunoglobulin genes (see *e.g.,* Taylor *et al.* (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of particular immunoglobulin gene sequences (such as human immunoglobulin gene sequences) to other DNA sequences. Examples of recombinant antibodies include chimeric, CDR-grafted and humanized antibodies.

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies used in the invention may include amino acid residues not encoded by human germline immunoglobulin sequences *(e.g.,* mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

Recombinant human antibodies of the invention have variable regions, and may also include constant regions, derived from human germline immunoglobulin sequences (See Kabat *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). In certain embodiments, however, such recombinant human antibodies are subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo. In certain embodiments, however, such recombinant antibodies are the result of selective mutagenesis or backmutation or both.

The term "backmutation" refers to a process in which some or all of the somatically mutated amino acids of a human antibody are replaced with the corresponding germline residues from a homologous germline antibody sequence. The heavy and light chain

sequences of a human antibody of the invention are aligned separately with the germline sequences in the VBASE database to identify the sequences with the highest homology. Differences in the human antibody of the invention are returned to the germline sequence by mutating defined nucleotide positions encoding such different amino acid. The role of each amino acid thus identified as candidate for backmutation should be investigated for a direct or indirect role in antigen binding and any amino acid found after mutation to affect any desirable characteristic of the human antibody should not be included in the final human antibody. To minimize the number of amino acids subject to backmutation those amino acid positions found to be different from the closest germline sequence but identical to the corresponding amino acid in a second germline sequence can remain, provided that the second germline sequence is identical and colinear to the sequence of the human antibody of the invention for at least 10, preferably 12 amino acids, on both sides of the amino acid in question. Backmuation may occur at any stage of antibody optimization.

The term "chimeric antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species and constant region sequences from another species, such as antibodies having murine heavy and light chain variable regions linked to human constant regions.

The term "CDR-grafted antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species but in which the sequences of one or more of the CDR regions of VH and/or VL are replaced with CDR sequences of another species, such as antibodies having murine heavy and light chain variable regions in which one or more of the murine CDRs *(e.g.,* CDR3) has been replaced with human CDR sequences.

The term "humanized antibody" refers to an antibody that comprises heavy and light chain variable region sequences from a non-human species (e.g., a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like", *i.e.,* more similar to human germline variable sequences. One type of humanized antibody is a CDR-grafted antibody, in which human CDR sequences are introduced into non-human VH and VL sequences to replace the corresponding nonhuman CDR sequences. Also a "humanized antibody" is an antibody or a variant, derivative, analog or fragment thereof that specifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody.

An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities *(e.g.,* an isolated antibody that specifically binds the p40 subunit of IL-12/IL-23 is substantially free of antibodies that specifically bind antigens other than the p40 subunit of IL-12/IL-23). An isolated antibody that specifically binds a target, *e.g.,* the p40 subunit of IL-12/IL-23, may, however, have cross-reactivity to other antigens, such as, *e.g.,* the p40 subunit of IL-12/IL-23 molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

A "neutralizing antibody" as used herein is intended to refer to an antibody whose binding to antigen results in inhibition of the biological activity of the antigen *(e.g.,* IL-12/IL-23, amyloid beta). This inhibition of the biological activity of the antigen can be assessed by measuring one or more indicators of the antigen's biological activity. For example, indicators of the biological activity of human IL-12 (hIL-12) can be assessed by one or more of several standard *in vitro* or *in vivo* assays known in the art, such as inhibition of human phytohemagglutinin blast proliferation in a phytohemagglutinin blast proliferation assay (PHA), or inhibition of receptor binding in a human IL-12 receptor binding assay (see Example 3-Interferon-gamma Induction Assay of U.S. Patent No. 6,914,128, hereby incorporated by reference in its entirety).

The term "activity" includes activities such as the binding specificity/affinity of an antibody for an antigen, for example, an anti-hIL-12 antibody that binds to an IL-12 antigen and/or the neutralizing potency of an antibody, for example, an anti-hIL-12 antibody whose binding to hIL-12 inhibits the biological activity of hIL-12, *e.g.* inhibition of PHA blast proliferation or inhibition of receptor binding in a human IL-12 receptor binding assay (see Example 3 of U.S. Patent No. 6,914,128).

The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.). For further descriptions, see Jonsson, U., *et al.* (1993) Ann. Biol. Clin. 51:19-26; Jonsson, U., *et al.* (1991) Biotechniques 11:620-627; Johnsson, B., *et al.* (1995) J. Mol. Recognit. 8:125-131; and Johnnson, B., *et al.* (1991) Anal. Biochem. 198:268-277.

The term "K_{on}", as used herein, is intended to refer to the on rate constant for association of a binding protein *(e.g.*, an antibody) to the antigen to form the, *e.g.*, antibody/antigen complex as is known in the art.

The term "$\mathbf{K_{off}}$", as used herein, is intended to refer to the off rate constant for dissociation of an antibody from the antibody/antigen complex.

The term "Kd", as used herein, is intended to refer to the dissociation constant of a particular antibody-antigen interaction and refers to the value obtained in a titration measurement at equilibrium, or by dividing the dissociation rate constant ($\mathbf{k_{off}}$) by the association rate constant (k_{on}).

The phrase "human interleukin 12" or "human IL-12" (abbreviated herein as hIL-12, or IL-12), as used herein, includes a human cytokine that is secreted primarily by macrophages and dendritic cells. The term includes a heterodimeric protein comprising a 35 kD subunit (p35) and a 40 kD subunit (p40) which are both linked together with a disulfide bridge. The heterodimeric protein is referred to as a "p70 subunit". The structure of human IL-12 is described further in, for example, Kobayashi, *et al.* (1989) *J. Exp Med.* 170:827-845; Seder, *et al.* (1993) *Proc. Natl. Acad. Sci.* 90:10188-10192; Ling, *et al.* (1995) *J. Exp Med.* 154:116-127; Podlaski, *et al.* (1992) *Arch. Biochem. Biophys.* 294:230-237; and Yoon *et al.* (2000) *EMBO Journal* 19(14): 3530-3541. The term "human IL-12" is intended to include recombinant human IL-12 (rh IL-12), which can be prepared by standard recombinant expression methods.

The phrase "human interleukin 23" or "human IL-23" (abbreviated herein as hIL-23, or IL-23), as used herein, includes a human cytokine that is secreted primarily by macrophages and dendritic cells. The term includes a heterodimeric protein comprising a 19 kD subunit (pi 9) and a 40kD subunit (p40) which are both linked together with a disulfide bridge. The heterodimeric protein is referred to as a "p40/p 19" heterodimer. The structure of human IL-23 is described further in, for example, Beyer *et al.* (2008) *J. Mol. Biol.* 382:942-955; Lupardus *et al.* (2008) *J. Mol. Biol.* 382:931-941. The term "human IL-23" is intended to include recombinant human IL-23 (rhIL-23), which can be prepared by standard recombinant expression methods.

The phrase "p40 subunit of human IL-12/IL-23" or "p40 subunit of human IL-12 and/or IL-23," or "p40 subunit" as used herein, is intended to refer to a p40 subunit that is shared by human IL-12 and human IL-23. The structure of the p40 subunit of IL-12/IL-23 is

described in, for example, Yoon *et al.* (2000) *EMBO Journal* 19(14): 3530-3541.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

Various aspects of the invention are described in further detail in the following subsections.

II. Compositions of the Invention

The present invention features protein compositions and protein pharmaceutical formulations *(e.g.,* antibody compositions and formulations) having improved properties as compared to art-recognized compositions and formulations. Protein aggregation is a common problem in protein solutions, and often results from increased concentration of the protein. The instant invention provides compositions containing proteins which are substantially free of protein aggregation. The instant invention also provides reformulated compositions containing proteins which have substantially reduced amounts of protein aggregation.

The present invention is based on the surprising finding that for protein compositions having protein concentrations below the RSC, reductions in polyol concentrations leads to significantly reduced protein aggregation after long-term storage. Thus, by reducing the level of polyol, *e.g.,* a sugar alcohol, or eliminating the polyol content in a protein formulation with a protein concentration below the reverse stability protein concentration for the formulation,

the stability of a protein *(e.g.,* an antibody, or antigen-binding fragment thereof) in a formulation can be increased. In some embodiments, the formulations of the invention maintain a low level of protein aggregation and/or fragmentation *(e.g.,* less than 2% or less than 1%). Furthermore, the formulations of the invention, *e.g.,* antibody formulations with antibody concentrations below the reverse stability concentration, maintain solubility and maintain a low viscosity suitable for subcutaneous injection. In one embodiment, the formulations remain stable for extended periods of time *(e.g.,* 6 months, 12 months, 18 months or 24 months) and / or at elevated temperatures *(e.g.,* at 5°C, 25°C or 40°C). In one embodiment, the formulation comprises less than 14% protein aggregation after 6 months at elevated temperature *(e.g.,* at 40°C). In one embodiment, the formulations comprise less than 2.0% protein aggregation after 12 months at 5°C. In another embodiment, the formulations comprise less than 0.6% protein aggregation after 12 months at 5°C. In another embodiment, turbidity of the formulations is less than 100 NTU after a standard 48 hour stir-stress assay. Thus, the protein formulations with protein concentrations below the reverse stability concentration of the invention overcome a number of known challenges for formulations, including stability, viscosity, turbidity, and physical degradation challenges.

Generally, aggregation increases as the protein concentration increases (Shire SJ, Shahrokh Z, Liu J. 2004. Challenges in the development of high protein concentration). Protein aggregation is generally concentration dependent. The mean-field lattice model predicts that proteins will aggregate at sufficiently high concentrations (Fields *et al.,* J. Phys. Chem. 96, 3974-3981 (1992)). Such an increase is almost always counteracted by adding polyol excipients, *e.g.,* sugar alcohol, which is known in the art to reduce protein aggregation in protein composition in general. Increased turbidity is often associated with the formation of insoluble protein aggregates, precipitates, or protein particles *(e.g.,* aggregation). The present invention is based on the surprising discovery that protein concentrations below the reverse stability concentration, the addition of polyol excipients can lead to greater amounts of protein aggregation, and not reduced amounts.

In one embodiment, formulations of the invention include protein concentrations below the reverse stability concentration such that the liquid formulation does not show significant opalescence, aggregation, or precipitation.

The methods of the invention provide a means of formulating a protein at protein concentrations below the reverse stability concentration with reduced amounts of excipients, or without excipients, that destabilize proteins at those concentrations. In particular, the

methods of the invention provide a means of increasing the protein stability at protein concentrations below the reverse stability concentration by reducing the amount of polyol in protein formulations. The concentration of the protein in the formulations obtained using the methods of the invention can be any amount in accordance with the desired concentration. For example, the concentration of protein in a formulation made according to the methods herein is less than about 10 μg/mL; less than about 1 mg/mL; less than about 10 mg/mL; less than about 20 mg/mL; less than about 50 mg/mL; less than about 75 mg/mL; less than about 100 mg/mL; less than about 110 mg/mL; less than about 120 mg/mL; less than about 125 mg/mL; less than about 130 mg/mL; less than about 135 mg/mL; less than about 140 mg/mL; less than about 150 mg/mL; less than about 175 mg/mL; less than about 200 mg/mL; less than about 220 mg/mL; less than about 250 mg/mL; less than about 300 mg/mL; or less than about 300 mg/mL. Ranges intermediate to the above recited concentrations, *e.g.,* less than about 113 mg/mL, less than about 214 mg/mL, and less than about 300 mg/mL, are also intended to be encompassed by the invention. In addition, ranges of values using a combination of any of the above recited values (or values between the ranges described above) as upper and/or lower limits are intended to be included, *e.g.,* 100 to 125 mg/mL, 113 to 125 mg/mL, 125 to 135 mg/mL, 127 to 133 mg/mL, and 126 to 200 mg/mL or more.

In several embodiments, the methods of the invention provide a means of increasing the protein concentration in protein formulations having protein concentrations below the reverse stability concentration without raising aggregations levels, by simultaneously reducing the amount of polyol in the protein formulations. By lowering the polyol content of a protein formulation while raising the protein concentration to a target concentration below the reverse stability concentration, the level of aggregates in the protein formulation with the higher protein concentration can be maintained or even lowered in comparison to the level of aggregates in the original protein formulation of lower protein concentration after longer-term storage or conditions of accelerated stability testing.

In various embodiments, the formulations of the invention comprise a protein concentration below the reverse stability concentration of an antibody, or antigen-binding portion thereof *(e.g.,* Briakinumab®, Stelara®, Enbrel®, Avastin®, Herceptin®, Erbitux®, Remicade®, or Simponi®). Such formulations comprise the antibody, or antigen-binding portion thereof, at concentrations including, for example, a concentration of about 100 mg/mL, about 150 mg/mL or about 200 mg/mL of an antibody, or antigen-binding fragment thereof. Accordingly, in one aspect of the invention the pharmaceutical formulation

comprises an antibody concentration of about 100 mg/mL. In another aspect of the invention the pharmaceutical formulation comprises an antibody concentration of about 150 mg/mL. In yet another aspect of the invention the pharmaceutical formulation comprises an antibody concentration of about 200 mg/mL.

In one aspect of the invention the pharmaceutical formulation comprises an antibody concentration below the Reverse Stability Concentration (RSC) of that protein in the formulation. In some embodiments, the formulations are low ionic formulations, *e.g.,* comprise a buffer and do not comprise additional ionic excipient(s), such as NaCl. In one embodiment of the invention the pharmaceutical formulation comprises an antibody concentration of less than about 200mg/mL, less than about 150 mg/mL, less than about 140 mg/mL, or less than about 130 mg/mL. Although the preferred embodiments of the invention are formulations comprising protein concentrations below the RSC, it is also contemplated that the formulations of the invention may comprise an antibody concentration between about 1 mg/mL and about 300 mg/mL, between about 50 mg/mL-250 mg/mL, between about 50 mg/mL- 150 mg/mL, or between about 50mg/mL-130 mg/mL. Concentrations and ranges intermediate to the above recited concentrations are also intended to be part of this invention *(e.g.,* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299 and 300 mg/mL).

The formulations of the invention provide the advantage that the resulting formulation has a low percentage of protein aggregates. In one embodiment, formulations of the

invention comprising a reduced amount of polyol, or no polyol, and a protein *(e.g.,* antibodies) concentration below the RSC for the formulation, contain less than about 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1.9%, 1.8%, 1.7%, 1.6%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2% or 0.1% protein aggregates. In one embodiment, the formulation comprises no more than about 7% aggregate protein; the formulation comprises no more than about 5% aggregate protein; the formulation comprises no more than about 4% aggregate protein; the formulation comprises no more than about 3% aggregate protein; the formulation comprises no more than about 2% aggregate protein; or the formulation comprising no more than about 1% aggregate protein. In one embodiment, the formulation comprises at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% monomer protein. Ranges intermediate to the above recited concentrations, *e.g.,* at least about 98.6%, no more than about 4.2%, are also intended to be part of this invention. In addition, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included.

Many protein-based pharmaceutical products need to be formulated at high concentrations. For example, antibody-based products increasingly tend to exceed 100 mg/mL in their Drug Product (DP) formulation to achieve appropriate efficacy and meet a typical patient usability requirement of a maximal ~1 mL injection volume. Accordingly, downstream processing steps, such as diafiltration into the final formulation buffer or ultrafiltration to increase the protein concentration, are also conducted at higher concentrations.

In another aspect, the invention provides a composition comprising excipients, a surfactant, and a buffer system, in amounts sufficient to formulate a therapeutic protein, *e.g.,* an antibody, *e.g.,* briakinumab, for therapeutic use at a concentration of about, for example, 100 mg/mL. In one embodiment, the pharmaceutical compositions do not comprise polyol. In one embodiment, the pharmaceutical compositions comprise an amount of polyol that is reduced in comparison with a standard composition. In one embodiment, the pharmaceutical compositions comprise an amount of polyol that does not cause or contribute to protein instability, *e.g.,* protein aggregation and/or fragmentation.

In one aspect, the invention provides a pharmaceutical composition comprising a therpeutic protein, *e.g.,* an antibody or antigen binding fragment thereof, *(e.g.,* briakinumab), without the addition of polyol, in amounts sufficient to formulate a protein for therapeutic use.

In another aspect, the invention provides a pharmaceutical composition comprising a therapeutic protein, *e.g.,* an antibody or antigen binding fragment thereof *(e.g.,* briakinumab), formulated to have low concentrations of polyol *(e.g.,* where the protein concentration is below the reverse stability concentration for the protein in the formulation), in amounts sufficient to formulate a protein for therapeutic use.

The present invention also provides formulations comprising a therapeutic protein, e.g, an antibody or antigen-binding fragment thereof *(e.g.,* Briakinumab®, Stelara®, Enbrel®, Avastin®, Herceptin®, Erbitux®, Remicade®, or Simponi®) and having a turbidity of less than about 60 NTU after a standard 24 hour stir-stress assay, with reduced or low levels of polyol or without the addition of polyol *(e.g.,* about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37. 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, or 63 NTU). In another aspect, the invention provides formulations comprising a therapeutic protein, e.g, an antibody or antigen-binding fragment thereof *(e.g.,* Briakinumab®, Stelara®, Enbrel®, Avastin®, Herceptin®, Erbitux®, Remicade®, or Simponi®) having a turbidity of less than about 100 NTU after a standard 48 hour stir-stress assay, with reduced or low levels of polyol or without the addition of polyol *(e.g.,* about 35, 36, 37. 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 NTU). In yet another aspect, the invention provides formulations comprising a therapeutic protein, e.g, an antibody or antigen-binding fragment thereof *(e.g.,* Briakinumab®, Stelara®, Enbrel®, Avastin®, Herceptin®, Erbitux®, Remicade®, or Simponi®) having a turbidity of less than about 40 NTU after 3 months storage at 5°C, 25°C, or 40°C, with reduced or low levels of polyol or without the addition of polyol *(e.g.,* about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37. 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60 NTU).

In certain embodiments of the invention, a formulation is prepared comprising the protein in a pH-buffered solution. The buffer of this invention has a pH ranging from about 4 to about 8, preferably from about 4.5 to about 7.0, more preferably from about 4.5 to about 6.0, even more preferably from about 4.8 to about 5.5, and most preferably has a pH of about 5.0 to about 6.4. In one embodiment, the pH of the formulation of the invention is about 5.2. In another embodiment, the pH of the formulation of the invention is about 6.0. Ranges intermediate to the above recited pH's are also intended to be part of this invention *(e.g.,* 4.5,

4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4). Ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included, *e.g.,* 5.2 - 5.8. Examples of buffers that will control the pH within this range include phosphate, acetate *(e.g.* sodium acetate), succinate (such as sodium succinate), gluconate, glutamate, histidine, citrate and other organic acid buffers. In one embodiment, the buffer is histidine.

In a particular embodiment of the invention, the formulation comprises a buffer system which contains citrate and/or phosphate to maintain the pH in a range of about 5.0 to about 6.4. In one embodiment, the pH of the formulation is about 5.2. In another embodiment, the pH of the formulation is about 6.0.

In another preferred embodiment, the buffer system includes citric acid monohydrate, sodium citrate, disodium phosphate dihydrate, and/or sodium dihydrogen phosphate dihydrate. However, the surprising findings of this invention are the consequence of protein-polyol interactions or the interaction of effects exerted on the protein by the presence of polyol in the composition , *e.g.,* effects on activity, osmolarity, density, etc. Thus, the invention applies to protein formulations for any pH value at which the protein is formulated.

A detergent or surfactant may also be added to the therapeutic protein formulation of the invention. Exemplary detergents include nonionic detergents such as polysorbates *(e.g.* polysorbates 20, 80, etc.) or poloxamers *(e.g.* poloxamer 188). The amount of detergent added is such that it reduces aggregation of the formulated therapeutic protein and/or minimizes the formation of particulates in the formulation and/or reduces adsorption. In a preferred embodiment of the invention, the formulation includes a surfactant which is a polysorbate. In another preferred embodiment of the invention, the formulation contains the detergent polysorbate 80. In one preferred embodiment, the formulation contains between about 0.1 and about 2.0 mg/mL of polysorbate 80, *e.g.,* about 1 mg/mL.

Values and ranges intermediate to the above recited concentrations are also intended to be part of this invention, *e.g.,* 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9. In addition, ranges of values using a combination of any of the above-recited values as upper and/or lower limits are intended to be included, *e.g.,* 0.3 to 1.1 mg/mL.

In one embodiment, the formulation of the invention consists essentially of a therapeutic protein, e.g, an antibody or antigen-binding fragment thereof, at a concentration of at least about 50 mg/mL or about 100 mg/mL, wherein the concentration is below the RSC, a surfactant *(e.g.,* polysorbate 80), a buffering system, and a reduced or low level of polyol.

In one embodiment, the formulation contains any one or more of the above-identified agents and is essentially free of preservatives, such as benzyl alcohol, phenol, m-cresol, chlorobutanol and benzethonium CI. In another embodiment, a preservative may be included in the formulation. One or more other pharmaceutically acceptable carriers, excipients or stabilizers such as those described in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980) may be included in the formulation provided that they do not significantly adversely affect the desired characteristics of the formulation. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed and include; additional buffering agents; co-solvents; antioxidants including ascorbic acid and methionine; chelating agents such as EDTA; metal complexes *(e.g.* Zn-protein complexes); biodegradable polymers such as polyesters; and/or salt-forming counterions such as sodium.

The formulation herein may also be combined with one or more other therapeutic agents as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect the therapeutic protein of the formulation. Such therapeutic agents are suitably present in combination in amounts that are effective for the purpose intended. Additional therapeutic agents which can be combined with the formulation of the invention are further described in U.S. Pat. Nos. 6,090,382, 6,258,562, and 6,914,128, each of which is incorporated herein by reference.

The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes prior to, or following, preparation of the formulation, or other methods know to those skilled in the art.

In one embodiment, the compositions or formulations of the invention are liquid compositions or formulations. The liquid compositions/formulations of the invention have advantageous stability and storage properties. Compositions/formulations of the invention include, but are not limited to, formulations which are frozen, lyophilized, spray-dried, or formulations in which the active ingredient is suspended. Stability can be measured at a selected temperature for a selected time period. In one aspect of the invention, the protein in the liquid formulations is stable in a liquid form for at least about 3 months; at least about 4 months, at least about 5 months; at least about 6 months; at least about 12 months; at least about 18 months; at least 24 months; at least 36 months; at least 48 months; at least 60 months. Values and ranges intermediate to the above recited time periods are also intended to be part of this invention, *e.g.,* about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46,

47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 and 60 months. In addition, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. Preferably, the formulation is stable at room temperature (about 30°C) or at 40°C for at least about 1 month and/or stable at about 2-8°C for at least about 1 year, or more preferably stable at about 2-8°C for at least about 2 years. Furthermore, the formulation is preferably stable following freezing (to, *e.g.,* -80°C) and thawing of the formulation, hereinafter referred to as a "freeze/thaw cycle."

Stability of a protein in a liquid formulation may also be defined as the percentage of monomer, aggregate, or fragment, or combinations thereof, of the protein in the formulation. A protein "retains its physical stability" in a formulation if it shows substantially no signs of aggregation, precipitation and/or denaturation, upon visual examination of color and/or clarity or as measured by UV light scattering or by size exclusion chromatography. In one aspect of the invention, a stable liquid formulation is a formulation having less than about 10%, less than about 5%, and preferably less than about 2% of the protein being present as aggregate in the formulation.

In one embodiment, the physical stability of a liquid formulation is determined by determining turbidity of the formulation following a stir stress assay, *e.g.,* 24 hour or 48-hour stir-stress assay. For example, a stir stress assay may be performed by placing a suitable volume of a liquid formulation in a beaker with a magnetic stirrer, *e.g.,* (multipoint HP, 550 rpm), removing aliquots at any suitable time, *e.g.,* at T0-T48 (hrs), and performing suitable assays as desired on the aliquots. Samples of a formulation under the same conditions but without stirring serve as control.

Turbidity measurements may be performed using a laboratory turbidity measurement system from Hach (Germany) and are reported as nephelometric units (NTU).

The concentration of protein in the formulations of the invention is not limited by the protein size and the formulation may include any size range of proteins. Included within the scope of the invention is a formulation comprising at least 50 mg/mL and as much as 200 mg/mL or more of a protein, which may range in size from 5 kDa to 150 kDa or more. In one embodiment, the protein in the formulation of the invention is at least about 15 kD in size, at least about 20 kD in size; at least about 47 kD in size; at least about 60 kD in size; at least about 80 kD in size; at least about 100 kD in size; at least about 120 kD in size; at least about 140 kD in size; at least about 160 kD in size; or greater than about 160 kD in size. Ranges intermediate to the above recited sizes are also intended to be part of this invention. In

addition, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included.

The formulation herein may also contain more than one protein. With respect to pharmaceutical formulations, an additional, distinct protein may be added as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect the other protein. For example, it may be desirable to provide two or more antibodies which bind to a single antigen *(e.g.,* IL-12/23) in a single formulation. Furthermore, two or more antibodies that bind to different antigens *(e.g.,* anti-IL12/23) may be combined in the one formulation. Such proteins are suitably present in combination in amounts that are effective for the purpose intended.

Examples of proteins that may be included in the composition or formulation include antibodies, or antigen-binding fragments thereof. Examples of different types of antibodies, or antigen-binding fragments thereof, that may be used in the invention include, but are not limited to, a chimeric antibody, a human antibody, a humanized antibody, and a domain antibody (dAb). In one alternative, the protein is a therapeutic protein. Examples of proteins which may be included in the methods and compositions described herein, include mammalian proteins, including recombinant proteins thereof. Proteins suitable for use in the compositions and formulations of the invention are discussed in further detail below in Section IV.

Turbidity measurements may be performed using a laboratory turbidity measurement system from Hach (Germany) and are reported as nephelometric units (NTU).

The liquid formulations of the invention also have advantageous tolerability properties. Tolerability is evaluated based on assessment of subject-perceived injection site pain using the Pain Visual Analog Scale (VAS).

A (VAS) is a measurement instrument that measures pain as it ranges across a continuum of values, *e.g.,* from none to an extreme amount of pain. Operationally a VAS is a horizontal line, about 100 mm in length, anchored by numerical and/or word descriptors, *e.g.,* 0 or 10, or 'no pain' or 'excruciating pain', optionally with additional word or numeric descriptors between the extremes, *e.g.,* , mild, moderate, and severe; or 1 through 9) (see, *e.g.,* Lee JS, *et al.* (2000) Acad Emerg Med 7:550).

Additional indicators of tolerability that may be measured include, for example, the Draize Scale (hemorrhage, petechiae, erythema, edema, pruritus) and bruising.

III. Methods of the Invention

In one aspect of the invention, a method for formulating a stable protein formulation with a protein concentration below the reverse stability concentration is provided.

The invention is based on the surprising discovery that at some concentrations of protein in some formulations, commonly found components of protein formulations can have a deleterious effect on the protein of the formulation. For example, polyols, *e.g.,* sugars and sugar alcohols, are commonly known in the art to enhance protein stability, by reducing protein aggregation and destabilization, thereby reducing turbidity and protein fragmentation. Applicants have discovered that polyols can actually have the opposite effect on protein stability, *e.g.,* they can increase aggregation, turbidity and fragmentation at a variety of formulation conditions of frequent use with the pharmaceutical compositions.

As noted above, the formation of non-native protein aggregates is considered to be a two-step process. The first step is the occurrence of a permutation of protein structure, *e.g.,* a partial unfolding of the protein. The second step is the association of two protein molecules with structural permutations to form an aggregated molecule. This process of the formation of non-native protein aggregates can be rate-limited by either the first step or the second step, which depends on a number of factors, including protein concentration, excipient concentration, and protein identity. At low protein concentrations, it is the second step *(i.e.,* the associate step) that is the rate limiting step, whereas it is at higher protein concentrations that the first step *(i.e.,* the unfolding step) is rate-limiting. Applicants have discovered that at certain protein concentrations *(i.e.,* protein concentrations below the RSC) in protein formulations, polyols act as crowders, driving protein molecules together and promoting the second step of the aggregation process. Thus, in these formulations a higher concentration of polyols increases the crowding effect and accelerates the aggregate formation process.

The destabilizing effect of polyols on proteins at protein concentrations below the reverse stability concentration can been seen in comparisons made between otherwise identical formulations containing different concentrations of polyol (as well as between formulations containing different concentrations of protein), as described in detail in the Examples herein. For example, for a protein formulation having a protein concentration of interest, *i.e.,* 100 mg/ml, incorporation of a polyol, *e.g.* sucrose at 80 mg/mL, at one concentration may provide a destabilizing effect, whereas incorporation of that same polyol at a higher protein concentration will have a stabilizing effect.

As noted above, variations in the concentration of polyol in a protein formulation, as well as variations in the concentration of protein in a protein formulation containing a polyol, can impact protein stability. Accordingly, one aspect of the invention features a method of determining the RSC for a protein in a particular formulation, *i.e.,* he protein concentration for a formulation below which the addition of a polyol (any polyol concentration of pharmaceutical interest and typically < 200 mg/ml) may be destabilizing for the protein formulation *(e.g.,* as detected by higher levels of aggregated protein in the formulation). Put in other words, the invention features a method of determining the concentration of a protein in a protein formulation containing a given polyol, below which the net stabilizing effect of the polyol is replaced with a net destabilizing effect *(e.g.,* as detected by higher levels of aggregated protein and/or protein fragments in the formulation). By determining the reverse stability concentration, the stability of a protein formulation can be improved *(e.g.,* a reduction in the protein aggregation and/or fragmentation of a formulation can be achieved), for example, by reducing the polyol concentration or eliminating the polyol from the formulation if the protein concentration is below the reverse stability concentration for the formulation. Thus, the stability of formulations with protein concentrations below the reverse stability concentration can be improved by reducing the polyol concentration. In one embodiment, the stability of formulations with protein concentrations below the reverse stability concentration can be improved by eliminating the polyol.

Accordingly, one aspect of the invention provides a method of determining, for a protein formulation containing a protein and a particular polyol, the reverse stability concentration for the protein in that formulation. As described in Figure 1A and B and examples herein, a group of two or more protein formulations comprising a buffer and having different protein concentrations *(e.g.,* two different protein concentrations equal to or greater than 100 mg/ml) are prepared, each identical except for having a different concentration of polyol. Each of the protein formulations is optionally assayed prior to a treatment phase to determine the content of monomer, aggregated and/or fragmented protein, as described below in the Examples *(e.g.,* Table 2A). Subsequently, each of the protein formulations undergoes a treatment phase. For example, each protein formulation may be stored for an extended period *(e.g.,* 12 months) at a specific temperature *(e.g.,* 40°C, or 5°C), and/or in the presence or absence of additional ionic excipients, *e.g.,* NaCl. In some embodiments, the protein formulations undergo a physical stress test such as the stir-stress assay described herein. In other embodiments, the protein formulations undergo cycles of freezing and thawing. In yet

other embodiments, samples of the same formulation receive differential treatment, *e.g.,* storage for a period of time in different temperatures. Following the treatment phase, the protein formulations are assayed to determine the content of protein monomer, aggregates and/or fragments. By comparing the results between formulations containing different concentrations of the polyol *(e.g.,* at one protein concentration, or between series of formulations at different protein concentrations, or in the presence and/or absence of additional ionic excipients, *e.g.,* NaCl) information regarding the reverse stability concentration can be obtained. In some embodiments, results between formulations are additionally compared for each protein formulation before and after treatment. The Reserve Stability Concentration of the protein can be identified by determing the protein concentration at which the presence of polyol has no net effect on the stability over a range of polyols.

As an example, Figures 1A and IB show that the level of protein aggregation is higher in the presence of polyols when compared to the formulation without polyol at 100 mg/mL. At 200 mg/ml protein concentration *(e.g.,* a high protein concentration for this protein in this formulation) the effect is reversed. Further, Figure 2 shows results at 40°C for two different proteins, ABT-736 in Figure 2A and ABT-874 in Figure 2B, formulated with a range of protein concentrations. The results clearly show that the addition of sugar is deleterious at concentrations below the reverse stability concentration *(e.g.,* where the slope of the line is zero or positive). The addition of sugars is only useful at higher concentrations above the RSC(i.e., where the slope of the line is negative).

In another aspect, the invention provides a method of determining the protein concentration for the protein *(i.e.,* the Reverse Stability Concentration) below which a polyol in any concentration of pharmaceutical interest is destabilizing for the formulation. As described above, a comparison of stability upon long-term storage of protein formulations and having the same polyol concentrations but varying protein concentrations, reveals both the stabilizing and destabilizing effects of polyols on protein formulations, as compared to each other, depending on the protein concentration. Thus, in another aspect, the invention provides methods of analyzing formulations with concentrations of protein and polyols in which the presence of polyols has either a stabilizing effect or a destabilizing effect, to determine the concentration of protein wherein the effect of the polyol is neutral *(i.e.,* the Reverse Stability Concentration).

IV. Proteins Suitable for Use in the Invention

Proteins which may be used in the compositions, formulations and methods of the invention may be any size, *i.e.*, molecular weight (M_w). For example, the protein may have a M_w equal to or greater than about 1 kDa, a M_w equal to or greater than about 10 kDa, a M_w equal to or greater than about 47 kDa, a M_w equal to or greater than about 57 kDa, a M_w equal to or greater than about 100 kDa, a M_w equal to or greater than about 150 kDa, a M_w equal to or greater than about 200 kDa, or a M_w equal to or greater than about 250 kDa. Numbers intermediate to the above recited M_w, *e.g.*, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73 , 74, 75, 76, 77, 78, 79, 80, 81, 82 , 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 153, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, and so forth, as well as all other numbers recited herein, are also intended to be part of this invention. Ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included in the scope of the invention. For example, proteins used in the invention may range in size from 57 kDa to 250 kDa, from 56 kDa to 242 kDa, from 60 kDa to 270 kDa, and so forth.

The methods of the invention include formulating protein compositions that comprise one protein, or at least two or more distinct proteins. For example, the protein composition may contain two or more types of antibodies directed to different molecules or different epitopes of the same molecule. In one embodiment, the protein composition may contain one therapeutic protein, *e.g.*, a therapeutic antibody, and one or more additional non-therapeutic proteins.

In one embodiment, the protein used in the composition is a therapeutic protein, including, but not limited to, fusion proteins and enzymes. Examples of therapeutic proteins include, but are not limited to, Pulmozyme (Dornase alfa), Regranex (Becaplermin), Activase (Alteplase), Aldurazyme (Laronidase), Amevive (Alefacept), Aranesp (Darbepoetin alfa), Becaplermin Concentrate, Betaseron (Interferon beta-1b), BOTOX (Botulinum Toxin Type A), Elitek (Rasburicase), Elspar (Asparaginase), Epogen (Epoetin alfa), Enbrel (Etanercept), Fabrazyme (Agalsidase beta), Infergen (Interferon alfacon-1), Intron A (Interferon alfa-2a), Kineret (Anakinra), MYOBLOC (Botulinum Toxin Type B), Neulasta (Pegfilgrastim), Neumega (Oprelvekin), Neupogen (Filgrastim), Ontak (Denileukin diftitox), PEGASYS

(Peginterferon alfa-2a), Proleukin (Aldesleukin), Pulmozyme (Dornase alfa), Rebif (Interferon beta-la), Regranex (Becaplermin), Retavase (Reteplase), Roferon-A (Interferon alfa-2), TNKase (Tenecteplase), and Xigris (Drotrecogin alfa), Arcalyst (Rilonacept), NPlate (Romiplostim), Mircera (methoxypolyethylene glycol-epoetin beta), Cinryze (CI esterase inhibitor), Elaprase (idursulfase), Myozyme (alglucosidase alfa), Orencia (abatacept), Naglazyme (galsulfase), Kepivance (palifermin) and Actimmune (interferon gamma- lb).

Other examples of proteins which may be included in the methods and compositions described herein, include mammalian proteins, including recombinant proteins thereof, such as, *e.g.,* growth hormone, including human growth hormone and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; a-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or tissue-type plasminogen activator (t-PA); bombazine; thrombin; tumor necrosis factor-cc and -β enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-α); serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin- associated peptide; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; an integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGFα and TGF-β, including TGF-β 1, TGF-β 2, TGF- β 3, TGF-.β 4, or TGF- β 5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(l-3)-IGF-I (brain IGF-I); insulin-like growth factor binding proteins; CD proteins such as CD3, CD4, CD8, CD19 and CD20; erythropoietin (EPO); thrombopoietin (TPO); osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-cc, - β., and -γ.; colony stimulating factors (CSFs), *e.g.,* M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.,* IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor (DAF); a viral antigen such as, for example, a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; immunoadhesins; antibodies; and biologically

active fragments or variants of any of the above-listed polypeptides.

The protein used in the invention may also be an antibody, or antigen-binding fragment thereof. In one embodiment, the antibody, or antigen-binding portion thereof, is a therapeutic antibody. Examples of antibodies that may be used in the invention include chimeric antibodies, non-human antibodies, human antibodies, humanized antibodies, and domain antibodies (dAbs).

In one embodiment, the antibody, or antigen-binding fragment thereof, is an anti-IL-12 or IL-23 antibody. Other examples of antibodies, or antigen-binding fragments thereof, which may be used in the methods and compositions of the invention include, but are not limited to, 1D4.7 (anti-IL-12/IL-23 antibody; Abbott Laboratories), 2.5(E)mgl (anti-IL-18; Abbott Laboratories), 13C5.5 (anti-IL-13 antibody; Abbott Laboratories), J695 (anti-IL-12/23, also referred to herein as "ABT-874" and/or "Briakinumab"; Abbott Laboratories), Campath (Alemtuzumab), CEA-Scan Arcitumomab (fab fragment), Erbitux (Cetuximab), Herceptin (Trastuzumab), Myoscint (Imciromab Pentetate), ProstaScint (Capromab Pendetide), Remicade (Infliximab), ReoPro (Abciximab), Rituxan (Rituximab), Simulect (Basiliximab), Synagis (Palivizumab), Verluma (Nofetumomab), Xolair (Omalizumab), Zenapax (Daclizumab), Zevalin (Ibritumomab Tiuxetan), Orthoclone OKT3 (Muromonab-CD3), Panorex (Edrecolomab), Mylotarg (Gemtuzumab ozogamicin), golimumab (Centocor), Cimzia (Certolizumab pegol), Soliris (Eculizumab), CNTO 1275 (ustekinumab), Vectibix (panitumumab), Bexxar (tositumomab and I^{131} tositumomab), an anti-IL-17 antibody Antibody 7 as described in International Application WO 2007/149032 (Cambridge Antibody Technology), the entire contents of which are incorporated by reference herein, the anti-IL-13 antibody CAT-354 (Cambridge Antibody Technology), the anti-human CD4 antibody CE9y4PE (IDEC-151, clenoliximab) (Biogen IDEC/Glaxo Smith Kline), the anti-human CD4 antibody IDEC CE9.1/SB-210396 (keliximab) (Biogen IDEC), the anti-human CD80 antibody IDEC-114 (galiximab) (Biogen IDEC), the anti-Rabies Virus Protein antibody CR4098 (foravirumab), and the anti-human TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-2) antibody HGS-ETR2 (lexatumumab) (Human Genome Sciences, Inc.), and Avastin (bevacizumab).

Techniques for the production of antibodies are provided below.

Polyclonal Antibodies

Polyclonal antibodies generally refer to a mixture of antibodies that are specific to a certain antigen, but bind to different epitopes on said antigen. Polyclonal antibodies are generally raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, *e.g.,* keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or R_1NCNR, where R and R_1 are different alkyl groups. Methods for making polyclonal antibodies are known in the art, and are described, for example, in *Antibodies: A Laboratory Manual,* Lane and Harlow (1988), incorporated by reference herein.

Monoclonal Antibodies

A "monoclonal antibody" as used herein is intended to refer to a hybridoma-derived antibody *(e.g.,* an antibody secreted by a hybridoma prepared by hybridoma technology, such as the standard Kohler and Milstein hybridoma methodology). For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al.* , Nature, 256:495(1975), or may be made by recombinant DNA methods (U.S. Pat. No. 4,816,567). Thus, a hybridoma-derived dual-specificity antibody of the invention is still referred to as a monoclonal antibody although it has antigenic specificity for more than a single antigen.

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.,* the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

In a further embodiment, antibodies can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al.,* Nature, 348:552-554 (1990). Clackson *et al.,* Nature, 352:624-628 (1991) and Marks *et al.,* J. Mol. Biol., 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al.,* Bio/Technology, 10:779-783 (1992)), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.,* Nuc. Acids. Res., 21:2265-2266 (1993)). Thus, these

techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

Antibodies and antibody fragments may also be isolated from yeast and other eukaryotic cells with the use of expression libraries, as described in U.S. Pat. Nos. 6,423,538; 6,696,251; 6,699,658; 6,300,065; 6,399,763; and 6,114,147. Eukaryotic cells may be engineered to express library proteins, including from combinatorial antibody libraries, for display on the cell surface, allowing for selection of particular cells containing library clones for antibodies with affinity to select target molecules. After recovery from an isolated cell, the library clone coding for the antibody of interest can be expressed at high levels from a suitable mammalian cell line.

Additional methods for developing antibodies of interest include cell-free screening using nucleic acid display technology, as described in U.S. Pat. Nos. 7,195,880; 6,951,725; 7,078,197; 7,022,479, 6,518,018; 7,125,669; 6,846,655; 6,281,344; 6,207,446; 6,214,553; 6,258,558; 6,261,804; 6,429,300; 6,489,116; 6,436,665; 6,537,749; 6,602,685; 6,623,926; 6,416,950; 6,660,473; 6,312,927; 5,922,545; and 6,348,315. These methods can be used to transcribe a protein *in vitro* from a nucleic acid in such a way that the protein is physically associated or bound to the nucleic acid from which it originated. By selecting for an expressed protein with a target molecule, the nucleic acid that codes for the protein is also selected. In one variation on cell-free screening techniques, antibody sequences isolated from immune system cells can be isolated and partially randomized polymerase chain reaction mutagenesis techniques to increase antibody diversity. These partially randomized antibody genes are then expressed in a cell-free system, with concurrent physical association created between the nucleic acid and antibody.

The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (U.S. Pat. No. 4,816,567; Morrison, *et al.,* Proc. Natl. Acad. Sci. USA, 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immuno globulin polypeptide.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

Chimeric or hybrid antibodies also may be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

Humanized Antibodies

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al,* Nature, 321:522-525 (1986); Riechmann *et al,* Nature, 332:323-327 (1988); Verhoeyen *et al,* Science, 239:1534-1536 (1988)), by substituting non-human *(e.g.,* rodent) CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some framework (FR) residues are substituted by residues from analogous sites in rodent antibodies. Additional references which describe the humanization process include Sims *et al,* J. Immunol., 151:2296 (1993); Chothia *et al,* J. Mol. Biol., 196:901 (1987); Carter *et al,* Proc. Natl. Acad. Sci. USA, 89:4285 (1992); Presta *et al,* J. Immunol., 151:2623 (1993), each of which is incorporated by reference herein.

In one embodiment, the formulations and methods of the invention comprise a humanized IgGl kappa monoclonal antibody, or antigen-binding fragment thereof, that selectively binds with high affinity to the extracellular domain of the human epidermal growth factor receptor 2 protein (HER2).

In another embodiment, the formulations and methods of the invention comprise a humanized IgGl kappa monoclonal antibody, or antigen-binding fragment thereof, that binds to and inhibits the biologic activity of human vascular endothelial growth factor.

Human antibodies

Alternatively, it is now possible to produce transgenic animals *{e.g.,* mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.,* Jakobovits *et al,* Proc. Natl. Acad. Sci. USA, 90:2551 (1993); Jakobovits *et al,* Nature, 362:255-258 (1993); Bruggermann *et ah,* Year in Immuno., 7:33 (1993). Human antibodies can also be derived from phage-display libraries (Hoogenboom *et al.,* J. Mol. Biol., 227:381 (1991); Marks *et al,* J. Mol. Biol., 222:581-597 (1991)).

In one embodiment, the formulation of the invention comprises an antibody, or antigen-binding portion thereof, which binds human IL-12, including, for example, the antibody J695 (Abbott Laboratories; also referred to as ABT-874) (U.S. Patent No. 6,914,128). J695 is a fully human monoclonal antibody designed to target and neutralize interleukin-12 and interleukin-23. In one embodiment, the antibody, or antigen-binding fragment thereof, has the following characteristics: it dissociates from human IL-lcc with a K_D of 3×10^{-7} M or less; dissociates from human IL-Iβ with a K_D of 5×10^{-5} M or less; and does not bind mouse IL-1a or mouse IL-1β. Examples and methods for making human, neutralizing antibodies which have a high affinity for human IL-12, including sequences of the antibody, are described in U.S. Patent No. 6,914,128, incorporated by reference herein.

In one embodiment, the formulation of the invention comprises an antibody, or antigen-binding portion thereof, which binds human IL-18, including, for example, the antibody 2.5(E)mgl (Abbott Bioresearch; also referred to as ABT-325) (see U.S. Patent Application No. 2005/0147610, incorporated by reference herein).

In one embodiment, the formulation of the invention comprises an anti-IL-12 / anti-IL-23 antibody, or antigen-binding portion thereof, which is the antibody 1D4.7 (Abbott Laboratories; also referred to as ABT-147) (see WO 2007/005608 A2, published Jan. 11, 2007, incorporated by reference herein).

In one embodiment, the formulation of the invention comprises an anti-IL-13 antibody, or antigen-binding portion thereof, which is the antibody 13C5.5 (Abbott

Laboratories; also referred to as ABT-308) (see. PCT/US2007/ 19660 (WO 08/127271), incorporated by reference herein).

In one embodiment, the formulation of the invention comprises an antibody, or antigen-binding portion thereof, which is the antibody 7C6, an anti-amyloid β antibody (Abbott Laboratories; see PCT publication WO 07/062852, incorporated by reference herein).

Bispecific Antibodies

Bispecific antibodies (BsAbs) are antibodies that have binding specificities for at least two different epitopes. Such antibodies can be derived from full length antibodies or antibody fragments *(e.g.,* F(ab')2 bispecific antibodies).

Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et ah,* Nature, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829 and in Traunecker *et al,* EMBO J., 10:3655-3659 (1991).

According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences.

The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CHI) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690 published Mar. 3, 1994. For further details of generating bispecific antibodies see, for example, Suresh *et ah,* Methods in Enzymology, 121:210 (1986).

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. The following techniques can also be used for the production of bivalent antibody fragments which are not necessarily bispecific. For example, Fab' fragments recovered from E. coli can be chemically coupled in vitro to form bivalent antibodies. See, Shalaby *et al,* J. Exp. Med., 175:217-225 (1992).

Various techniques for making and isolating bivalent antibody fragments directly from recombinant cell culture have also been described. For example, bivalent heterodimers have been produced using leucine zippers. Kostelny *et ah,* J. Immunol., 148(5): 1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. The "diabody" technology described by Hollinger *et al.,* Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific/bivalent antibody fragments. The fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming

two antigen-binding sites. Another strategy for making bispecific/bivalent antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber *et al.*, J. Immunol., 152:5368 (1994).

In one embodiment, the formulation of the invention comprises an antibody which is bispecific for IL-1 (including IL-lcc and IL-Iβ). Examples and methods for making bispecific IL-1 antibodies can be found in U.S. Provisional Appln. No. 60/878165, filed December 29, 2006.

In various embodiments, antibodies that can be used in the formulations of the invention include antibodies directed against the p40 subunit of IL-12 and/or IL-23, including the p40 subunit of human IL-12 and/or IL-23, and against amyloid beta. Specific examples of such antibodies are described in further detail below

(i) Antibodies directed against IL-12/IL-23

In one aspect of the invention, the formulation contains an antibody, *e.g.,* human antibody, that binds to an epitope of the p40 subunit of IL-12/IL-23. In one embodiment, the antibody binds to the p40 subunit when the p40 subunit is bound to the p35 subunit of IL-12. In one embodiment, the antibody binds to the p40 subunit when the p40 subunit is bound to the pl9 subunit of IL-23. In one embodiment, the antibody binds to the p40 subunit when the subunit is bound to the p35 subunit of IL-12 and also when the p40 subunit is bound to the pl9 subunit of Il-23. In a preferred embodiment, the antibody, or antigen-binding portion thereof, is an antibody like those described in U.S. Patent No. 6,914,128, the entire contents of which are incorporated by reference herein. For example, in a preferred embodiment, the antibody binds to an epitope of the p40 subunit of IL-12 to which an antibody selected from the group consisting of Y61 and J695, as described in U.S. Patent No. 6,914,128, binds. Especially preferred among the human antibodies is J695 as described in U.S. Patent No. 6,914,128. Other antibodies that bind IL-12 and/or IL-23 and which can be used in the formulations of the invention include the human anti-IL-12 antibody C340, as described in U.S. Patent No. 6,902,734, the entire contents of which are incorporated by reference herein.

In one embodiment, the formulation of the invention includes a combination of antibodies (two or more), or a "cocktail" of antibodies. For example, the formulation can include the antibody J695 and one or more additional antibodies.

In one aspect, the formulation of the invention contains J695 antibodies and antibody portions, J695-related antibodies and antibody portions, and other human antibodies and antibody portions with equivalent properties to J695, such as high affinity binding to hlL-12/IL-23 with low dissociation kinetics and high neutralizing capacity. For example, in one embodiment of the invention, the formulation contains a human antibody, or antigen-binding portion thereof, that dissociates from the p40 subunit of human IL-12/IL-23 with a K_d of 1.34 x 10^{-10} M or less or with a K_0ff rate constant of 1 x 10^{-3} s^{-}l or less, as determined by surface plasmon resonance. Preferably, the antibody, or antigen-binding portion thereof, dissociates from the p40 subunit of human IL-12/IL-23 with a k_{Q}ff rate constant of 1 x 10^{-4} s^{-1} or less, and more preferably with a k_{Q}ff rate constant of 1 x 10^{-}V ^{1} or less, or with a K_d of 1 x 10^{-10} M or less, and more preferably with a K_d of 9.74 x 10^{-11} M or less.

The dissociation rate constant (K_0f_f) of an IL-12/IL-23 antibody can be determined by surface plasmon resonance. Generally, surface plasmon resonance analysis measures real-time binding interactions between ligand (recombinant human IL-12 immobilized on a biosensor matrix) and analyte (antibodies in solution) by surface plasmon resonance (SPR) using the BIAcore system (Pharmacia Biosensor, Piscataway, NJ). Surface plasmon analysis can also be performed by immobilizing the analyte (antibodies on a biosensor matrix) and presenting the ligand (recombinant IL-12/IL-23 in solution) (see, for example, assays described in Example 5 of US 6,914,128, the contents of which are incorporated by reference herein). Neutralization activity of IL-12/IL-23 antibodies, or antigen binding portions thereof, can be assessed using one or more of several suitable *in vitro* assays (see for example, assays described in Example 3 of US 6,914,128, the contents of which are incorporated by reference herein).

In another embodiment of the invention, the formulation contains an antibody *(e.g.,* human antibody), or antigen-binding portion thereof, that neutralizes the biological activity of the p40 subunit of human IL-12/IL-23. In one embodiment, the antibody, or antigen-binding portion thereof, neutralizes the biological activity of free p40, *e.g.,* monomer p40 or a p40 homodimer, *e.g., a* dimer containing two identical p40 subunits. In preferred embodiments, the antibody, or antigen-binding portion thereof, neutralizes the biological activity of the p40 subunit when the p40 subunit is bound to the p35 subunit of 11-12 and/or when the p40 subunit is bound to the pl9 subunit of IL-23. In various embodiments, the antibody, or antigen-binding portion thereof, inhibits human IL-12-induced phytohemagglutinin blast proliferation

in an *in vitro* PHA assay with an IC_{50} of 1×10^{-7} M or less, preferably with an IC_{50} of 1×10^{-8} M or less, more preferably with an IC_{50} of 1×10^{-9} M or less, even more preferably with an IC_{50} of 1×10^{-10} M or less, and most preferably with an IC_{50} of 1×10^{-11} M or less. In other embodiments, the antibody, or antigen-binding portion thereof, inhibits human IL-12-induced human IFNy production with an IC_{50} of 1×10^{-10} M or less, preferably with an IC_{50} of 1×10^{-11} M or less, and more preferably with an IC_{50} of 5×10^{-12} M or less.

In yet another embodiment of the invention, the formulation contains a human antibody, or antigen-binding portion thereof, which has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1 and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2. In one embodiment, the human antibody, or antigen binding portion thereof, further has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3 and a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4. In one embodiment, the human antibody, or antigen binding portion thereof, further has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5 and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6. In a particularly preferred embodiment, the antibody, or antigen binding portion thereof, has heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8. The antibody, or antigen binding portion thereof, of the formulations of the invention can comprise a heavy chain constant region selected from the group consisting of IgGl, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions. Preferably, the antibody heavy chain constant region is IgGl. In various embodiments, the antibody, or antigen binding portion thereof, is a Fab fragment, a F(ab')2 fragment, or a single chain Fv fragment.

(ii) Antibodies directed against amyloid beta

In one aspect of the invention, the formulation contains an antibody, *e.g.*, humanized antibody, that binds to an epitope of the amyloid beta protein. In one embodiment, the antibody binds to oligomers of the amyloid beta protein. In one embodiment, the antibody binds to a epitope comprising the globulomer epitope of the amyloid beta protein, *e.g.*, Aβ(20-42), Aβ(20-40), Aβ(12-42), Aβ(12-40), Aβ(1-42) and Aβ(1-40). In a preferred embodiment, the antibody, or antigen-binding portion thereof, is an antibody like those described in U.S. Patent Application No. 2009/0175847 Al, the entire contents of which are incorporated by reference herein. For example, in a preferred embodiment, the antibody comprises an

antigen-binding domain which binds to amyloid-beta (20-42) globulomer, as described in Patent Application No. 2009/0175847 Al. Especially preferred among the humanized antibodies is 5F7, as described in Patent Application No. 2009/0175847 Al. Other antibodies that bind amyloid beta and which can be used in the formulations of the invention include the humanized anti-amyloid beta antibody 7C6, as described in U.S. Patent Application No. 2009/0175847 Al.

In one embodiment, the formulation of the invention includes a combination of antibodies (two or more), or a "cocktail" of antibodies. For example, the formulation can include the antibody 5F7 and one or more additional antibodies.

In one aspect, the formulation of the invention contains 5F7 antibodies and antibody portions, 5F7-related antibodies and antibody portions, and other humanized antibodies and antibody portions with equivalent properties to 5F7, such as high affinity binding to amyloid-beta with low dissociation kinetics and high neutralizing capacity. For example, in one embodiment of the invention, the formulation contains a humanized antibody, or antigen-binding portion thereof, that dissociates from the amyloid-beta (20-42) globulomer with a K_d of 1 x 10-6 M or less.

In another embodiment of the invention, the formulation contains an antibody *(e.g.,* human antibody), or antigen-binding portion thereof, that neutralizes the biological activity of the amyloid-beta. In various embodiments, the antibody, or antigen-binding portion thereof, binds to an Aβ(20-42) globulomer with a K_D in the range of 1×10^{-6} M to 1×10^{-12} M, more preferably with a K_D of 1×10^{-7} M or greater affinity, even more preferably with a K_D of 3×10^{-8} M or greater affinity, even more preferably with a K_D of 1×10^{-8} M or greater affinity, even more preferably with a K_D of 3×10^{-9} M or greater affinity, even more preferably with a K_D of 1×10^{-9} M or greater affinity, even more preferably with a K_D of 3×10^{-10} M or greater affinity, even more preferably with a K_D of 1×10^{-10} M or greater affinity, even more preferably with a K_D of 3×10^{-11} M or greater affinity, and most preferably with a K_D of 1×10^{-11} M or greater affinity.

In yet another embodiment of the invention, the formulation contains a human antibody, or antigen-binding portion thereof, which has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9 and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10. In one embodiment, the human antibody, or antigen binding portion thereof, further has a heavy chain CDR2 comprising the amino acid sequence of SEQ

ID NO: 11 and a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 12. In one embodiment, the human antibody, or antigen binding portion thereof, further has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13 and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14. In a particularly preferred embodiment, the antibody, or antigen binding portion thereof, has heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16. The antibody, or antigen binding portion thereof, of the formulations of the invention can comprise a heavy chain constant region selected from the group consisting of IgGl, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions. Preferably, the antibody heavy chain constant region is IgGl. In various embodiments, the antibody, or antigen binding portion thereof, is a Fab fragment, a F(ab')2 fragment, or a single chain Fv fragment.

V. Uses of the Invention

The compositions and formulations of the invention may be used both therapeutically or prophylactically, *i.e., in vivo,* or as reagents for *in vitro* or *in situ* purposes.

Therapeutic uses

The methods of the invention may also be used to make a stable protein formulation comprising a protein at a concentration below the reverse stability concentration and having characteristics which are advantageous for therapeutic use. The stable protein formulation having a protein concentration below the reverse stability concentration may be used as a pharmaceutical formulation to treat a disorder in a subject.

The formulation of the invention may be used to treat any disorder for which the therapeutic protein is appropriate for treating. A "disorder" is any condition that would benefit from treatment with the protein. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. In the case of an anti-IL-12/IL-23 antibody, a therapeutically effective amount of the antibody may be administered to treat a neurological disorder, such as multiple sclerosis,an autoimmune disease, such as rheumatoid arthritis, an intestinal disorder, such as Crohn's disease, or a skin disorder, such as psoriasis. Other examples of disorders in which the formulation of the invention may be used to treat include cancer, including breast cancer, leukemia, lymphoma, and colon cancer. There are numerous other examples of disorders in which IL-12 and/or IL-23 activity is detrimental. Examples in which IL-12 and/or IL-23

activity is detrimental are also described in U.S. Patent Nos. 6,914,128, the entire contents of which are incorporated herein by reference.

The term "subject" is intended to include living organisms, *e.g.,* prokaryotes and eukaryotes. Examples of subjects include mammals, *e.g.,* humans, dogs, cows, horses, pigs, sheep, goats, cats, mice, rabbits, rats, and transgenic non-human animals. In specific embodiments of the invention, the subject is a human.

The term "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder, as well as those in which the disorder is to be prevented.

The stable protein formulations comprising a protein at a concentration below the reverse stability concentration may be administered to a mammal, including a human, in need of treatment in accordance with known methods of administration. Examples of methods of administration include intravenous administration, such as a bolus or by continuous infusion over a period of time, intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, intradermal, transdermal, oral, topical, or inhalation administration.

A formulation of the invention can be administered to a human subject for therapeutic purposes (discussed further below). In one embodiment of the invention, the liquid pharmaceutical formulation is easily administratable, which includes, for example, a formulation which is self-administered by the patient. In a preferred embodiment, the formulation of the invention is administered through subcutaneous injection, preferably single use. Moreover, a formulation of the invention can be administered to a non-human mammal expressing a target antigen with which the antibody cross-reacts *(e.g.,* a primate, pig or mouse) for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of antibodies of the invention *(e.g.,* testing of dosages and time courses of administration).

One advantage of the formulations of the invention is the ability to prepare formulations comprising high concentrations of therapeutic protein without increasing the viscosity of the formulation. Therefore, the new formulations permit administration of high amounts *(e.g.,* effective amounts) of therapeutic protein in smaller volumes as compared to prior commercial formulations, thereby decreasing pain.

Another advantage of the formulation of the invention is that it may be used to deliver a high concentration of a protein, *e.g.,* a therapeutic protein, *e.g.,* an antibody, or antigen-

binding portion, *(e.g.,* briakinumab) to a subject subcutaneously. Thus, in one embodiment, formulations of the invention are delivered to a subject subcutaneously. In one embodiment, the subject administers the formulation to himself/herself.

Accordingly, in one embodiment, the stable protein formulations comprising a protein at a concentration below the reverse stability concentration is administered to the mammal by subcutaneous administration. For such purposes, the formulation may be injected using a syringe, as well as other devices including injection devices *(e.g.,* the Inject-ease and Genject devices); injector pens (such as the GenPen); needleless devices *(e.g.,* MediJector and Biojectorr 2000); and subcutaneous patch delivery systems. In one embdodiment, the device, *e.g.,* a syringe, autoinjector pen, contains a needle with a gauge ranging in size from 25 G or smaller in diameter. In one embodiment, the needle gauge ranges in size from 25G to 33 G (including ranges intermediate thereto, *e.g.,* 25sG, 26, 26sG, 27G, 28G, 29G, 30G, 31G, 32G, and 33G). In a preferred embodiment, the smallest needle diameter and appropriate length is chosen in accordance with the viscosity characteristics of the formulation and the device used to deliver the formulation of the invention.

One advantage of the methods/compositions of the invention is that they provide large concentrations of a protein in a solution which may be ideal for administering the protein to a subject using a needleless device. Such a device allows for dispersion of the protein throughout the tissue of a subject without the need for an injection by a needle. Examples of needleless devices include, but are not limited to, Biojectorr 2000 (Bioject Medical Technologies), Cool.Click (Bioject Medical Technologies), Iject (Bioject Medical Technologies), Vitajet 3, (Bioject Medical Technologies), Mhi500 (The Medical House PLC), Injex 30 (INJEX - Equidyne Systems), Injex 50 (INJEX - Equidyne Systems), Injex 100 (INJEX-Equidyne Systems), Jet Syringe (INJEX - Equidyne Systems), Jetinjector (Becton-Dickinson), J-Tip (National Medical Devices, Inc.), Medi-Jector VISION (Antares Pharma), MED-JET (MIT Canada, Inc.), DermoJet (Akra Dermojet), Sonoprep (Sontra Medical Corp.), PenJet (PenJet Corp.), MicroPor (Altea Therapeutics), Zeneo (Crossject Medical Technology), Mini-Ject (Valeritas Inc.), ImplaJect (Caretek Medical LTD), Intraject (Aradigm), and Serojet (Bioject Medical Technologies).

Also included in the invention are delivery devices that house the stable protein formulations comprising a protein at a concentration below the reverse stability concentration. Examples of such devices include, but are not limited to, a syringe, a pen (such as an autoinjector pen), an implant, an inhalation device, a needleless device, and a patch. An

example of an autoinjection pen is described in US Appln. No. 11/824516, filed June 29, 2007.

The invention also includes methods of delivering the formulations of the invention by inhalation and inhalation devices containing said formulation for such delivery. In one embodiment, the stable protein formulations comprising a protein at a concentration below the reverse stability concentration is administered to a subject via inhalation using a nebulizer or liquid inhaler. Generally, nebulizers use compressed air to deliver medicine as wet aerosol or mist for inhalation, and, therefore, require that the drug be soluble in water. Types of nebulizers include jet nebulizers (air-jet nebulizers and liquid-jet nebulizers) and ultrasonic nebulizers.

Examples of nebulizers include Akita™ (Activaero GmbH) (see US2001037806, EP1258264). Akita™ is a table top nebulizer inhalation system (Wt: 7.5 kg , BxWxH: 260 x 170 x 270) based on Pari's LC Star that provides full control over patient's breathing pattern. The device can deliver as much as 500 mg drug in solution in less than 10 min with a very high delivery rates to the lung and the lung periphery. 65% of the nebulized particles are below 5 microns and the mass median aerodynamic diameter (MMAD) is 3.8 microns at 1.8 bar. The minimum fill volume is 2 mL and the maximum volume is 8 mL. The inspiratory flow (200 mL/sec) and nebulizer pressure (0.3-1.8 bar) are set by the smart card. The device can be individually adjusted for each patient on the basis of a lung function test.

Another example of a nebulizer which may be used with compositions of the invention includes the Aeroneb® Go/Pro/Lab nebulizers (AeroGen). The Aeroneb® nebulizer is based on OnQ™ technology, *i.e.,* an electronic micropump (3/8 inch in diameter and wafer-thin) comprised of a unique dome-shaped aperture plate that contains over 1,000 precision-formed tapered holes, surrounded by a vibrational element. Aeroneb® Go is a portable unit for home use, whereas Aeroneb® Pro is a reusable and autoclavable device for use in hospital and ambulatory clinic, and Aeroneb® Lab is a device for use in pre-clinical aerosol research and inhalation studies. The features of the systems include optimization and customization of aerosol droplet size; low-velocity aerosol delivery with a precisely controlled droplet size, aiding targeted drug delivery within the respiratory system; flexibility of dosing; accommodation of a custom single dose ampoule containing a fixed volume of drug in solution or suspension, or commercially available solutions for use in general purpose nebulizers; continuous, breath-activated or programmable; and adaptable to the needs of a broad range of patients, including children and the elderly; single or multi-patient use.

Aerocurrent™ (AerovertRx corp) may also be used with compositions of the invention (see WO2006006963). This nebulizer is a portable, vibrating mesh nebulizer that features a disposable, pre-filled or user filled drug cartridge.

Staccato™ (Alexza Pharma) may also be used with compositions of the invention (see WO03095012). The key to Staccato™ technology is vaporization of a drug without thermal degradation, which is achieved by rapidly heating a thin film of the drug. In less than half a second, the drug is heated to a temperature sufficient to convert the solid drug film into a vapor. The inhaler consists of three core components: a heating substrate, a thin film of drug coated on the substrate, and an airway through which the patient inhales. The inhaler is breath-actuated with maximum dose delivered to be 20-25 mg and MMAD in the 1-2 micron range.

AERx® (Aradigm) may also be used with compositions of the invention (see W09848873, US5469750, US5509404, US5522385, US5694919, US5735263, US5855564). AERx® is a hand held battery operated device which utilizes a piston mechanism to expel formulation from the AERx® Strip. The device monitors patients inspiratory air flow and fires only when optimal breathing pattern is achieved. The device can deliver about 60% of the dose as emitted dose and 50-70% of the emitted dose into deep lung with <25% inter-subject variability.

Another example of a nebulizer device which may also be used with compositions of the invention includes Respimat® (Boehringer). Respimat® is a multi-dose reservoir system that is primed by twisting the device base, which is compressed a spring and transfers a metered volume of formulation from the drug cartridge to the dosing chamber. When the device is actuated, the spring is released, which forces a micro-piston into the dosing chamber and pushes the solution through a uniblock; the uniblock consists of a filter structure with two fine outlet nozzle channels. The MMAD generated by the Respimat® is 2 um, and the device is suitable for low dose drugs traditionally employed to treat respiratory disorders.

Compositions of the invention may also be delivered using the Collegium Nebulizer™ (Collegium Pharma), which is a nebulizer system comprised of drug deposited on membrane. The dosage form is administered to a patient through oral or nasal inhalation using the Collegium Nebulizer after reconstitution with a reconstituting solvent.

Another example of a nebulizer device which may also be used with compositions of the invention includes the Inspiration® 626 (Respironics). The 626 is a compressor based nebulizer for home care. The 626 delivers a particle size between 0.5 to 5 microns.

Nebulizers which can be used with compositions of the invention may include Adaptive Aerosol Delivery® technology (Respironics), which delivers precise and reproducible inhaled drug doses to patients regardless of the age, size or variability in breathing patterns of such patients. AAD® systems incorporate electronics and sensors within the handpiece to monitor the patient's breathing pattern by detecting pressure changes during inspiration and expiration. The sensors determine when to pulse the aerosol delivery of medication during the first part of inspiration. Throughout the treatment, the sensors monitor the preceding three breaths and adapt to the patient's inspiratory and expiratory pattern. Because AAD® systems only deliver medication when the patient is breathing through the mouthpiece, these devices allow the patient to take breaks in therapy without medication waste. Examples of AAD® system nebulizers include the HaloLite® AAD®, ProDose® AAD®, and I-Neb® AAD®.

The HaloLite® Adaptive Aerosol Delivery (AAD)® (Respironics) is a pneumatic aerosolisation system powered by a portable compressor. The AAD® technology monitors the patient's breathing pattern (typically every ten milliseconds) and, depending upon the system being used, either releases pulses of aerosolized drug into specific parts of the inhalation, or calculates the dose drawn during inhalation from a "standing aerosol cloud" (see EP 0910421, incorporated by reference herein).

The ProDos AAD® (Respironics) is a nebulizing system controlled by "ProDose Disc™" system. (Respironics). ProDos AAD® is a pneumatic aerosol system powered by a portable compressor, in which the dose to be delivered is controlled by a microchip-containing disc inserted in the system that, among other things, instructs the system as to the dose to deliver. The ProDose Disc™ is a plastic disc containing a microchip, which is inserted into the ProDose AAD® System and instructs it as to what dose to deliver, the number of doses, which may be delivered together with various control data including drug batch code and expiry date (see EP1245244, incorporated by reference herein). Promixin® can be delivered via Prodose AAD® for management of pseudomonas aeruginosa lung infections, particularly in cystic fibrosis. Promixin® is supplied as a powder for nebulization that is reconstituted prior to use.

The I-neb AAD® is a handheld AAD® system that delivers precise and reproducible drug doses into patients' breathing patterns without the need for a separate compressor ("I-Neb"). The I-neb AAD® is a miniaturized AAD® inhaler based upon a combination of electronic mesh-based aerosolisation technology (Omron) and AAD® technology to control

dosing into patients' breathing patterns. The system is approximately the size of a mobile telephone and weighs less than 8 ounces. I-neb AAD® has been used for delivery of Ventavis® (iloprost) (CoTherix / Schering AG).

Another example of a nebulizer which may be used with compositions of the invention is Aria™ (Chrysalis). Aria is based on a capillary aerosol generation system. The aerosol is formed by pumping the drug formulation through a small, electrically heated capillary. Upon exiting the capillary, the formulation rapidly cooled by ambient air to produce an aerosol with MMAD ranging from 0.5-2.0 um.

In addition the TouchSpray™ nebulizer (Odem) may be used to deliver a composition of the invention. The TouchSpray™ nebulizer is a hand-held device which uses a perforate membrane, which vibrates at ultrasonic frequencies, in contact with the reservoir fluid, to generate the aerosol cloud. The vibration action draws jets of fluid though the holes in the membrane, breaking the jets into drug cloud. The size of the droplets is controlled by the shape/size of the holes as well as the surface chemistry and composition of the drug solution. This device has been reported to deliver 83% of the metered dose to the deep lung. Details of the TouchSpray™ nebulizer are described in US Patent No, 6659364, incorporated by reference herein.

Additional nebulizers which may be used with compositions of the invention include nebulizers which are portable units which maximize aerosol output when the patient inhales and minimize aerosol output when the patient exhales using two one-way valves (see PARI nebulizers (PARI GmbH). Baffles allow particles of optimum size to leave the nebulizer. The result is a high percentage of particles in the respirable range that leads to improved drug delivery to the lungs. Such nebulizers may be designed for specific patient populations, such a patients less than three years of age (PARI BABY™) and nebulizers for older patients (PARI LC PLUS® and PARI LC STAR®).

An additional nebulizer which may be used with compositions of the invention is the e-Flow® nebulizer (PARI GmbH) which uses vibrating membrane technology to aerosolize the drug solution, as well as the suspensions or colloidal dispersions (, TouchSpray™ ;ODEM (United Kingdom)). An e-Flow® nebulizer is capable of handling fluid volumes from 0.5 ml to 5 ml, and can produce aerosols with a very high density of active drug, a precisely defined droplet size, and a high proportion of respirable droplets delivered in the shortest possible amount of time. Drugs which have been delivered using the e-Flow® nebulizer include

aztreonam and lidocaine. Additional details regarding the e-Flow® nebulizer are described in US 6962151, incorporated by reference herein.

Additional nebulizers which may be used with compositions of the invention include a Microair® electronic nebulizer (Omron) and a Mystic™ nebulizer (Ventaira). The Microair® nebulizer is extremely small and uses Vibrating Mesh Technology to efficiently deliver solution medications. The Microair device has 7 mL capacity and produces drug particle MMAD size around 5 microns. For additional details regarding the Microair® nebulizer see US patent publication no. 2004045547, incorporated by reference herein. The Mystic™ nebulizer uses strong electric field to break liquid into a spray of nearly monodispersed, charged particles. The Mystic™ system includes a containment unit, a dose metering system, aerosol generation nozzles, and voltage converters which together offer multi-dose or unit-dose delivery options. The Mystic™ device is breath activated, and has been used with Coras 1030™ (lidocaine HC1), Resmycin® (doxorubicin hydrochloride), Acuair (fluticasone propionate), NCE with ViroPharm, and NCE with Pfizer. Additional details regarding the Mystic™ nebulizer may be found in U.S. Patent No. 6397838, incorporated by reference herein.

Additional methods for pulmonary delivery of the formulation of the invention are provided in US Appln. No. 12/217,972, incorporated by reference herein.

The appropriate dosage ("therapeutically effective amount") of the protein will depend, for example, on the condition to be treated, the severity and course of the condition, whether the protein is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the protein, the type of protein used, and the discretion of the attending physician. The protein is suitably administered to the patient at one time or over a series of treatments and may be administered to the patient at any time from diagnosis onwards. The protein may be administered as the sole treatment or in conjunction with other drugs or therapies useful in treating the condition in question.

The formulations of the invention overcome the common problem of protein aggregation, and, therefore, provide a new means by which therapeutic proteins with low levels of protein aggregation may be administered to a patient.

In one embodiment, the effective amount of therapeutic protein may be determined according to a strictly weight based dosing scheme *(e.g.,* mg/kg) or may be a total body dose (also referred to as a fixed dose) which is independent of weight. In one example, an effective amount of the formulation is 0.8 mL of the formulation containing a total body dose of about

80 mg of therapeutic protein *(i.e.,* 0.8 mL of a 100 mg/mL therapeutic protein formulation of the invention). In another example, an effective amount of the formulation is 0.4 mL of the formulation of the invention containing a total body dose of about 40 mg of therapeutic protein *(i.e.,* 0.4 mL of a 100 mg/mL therapeutic protein formulation of the invention). In yet another example, an effective amount of the formulation is twice 0.8 mL of the formulation containing a total body dose of about 160 mg of therapeutic protein *(i.e.,* two units containing 0.8 mL each of a 100 mg/mL therapeutic protein formulation of the invention). In a further example, an effective amount of the formulation is 0.2 mL of the formulation of the invention containing a total body dose of about 20 mg of therapeutic protein *(i.e.,* 0.2 mL of a 100 mg/mL therapeutic protein formulation of the invention). Alternatively, an effective amount may be determined according to a weight-based fixed dosing regimen (see, *e.g.,* WO 2008/154543, incorporated by reference herein).

In one embodiment, a liquid pharmaceutical formulation of the invention may be administered to a subject via a prefilled syringe, an autoinjector pen, or a needle-free administration device. In one embodiment, the invention features a delivery device comprising a dose of the formulation comprising 100 mg/mL a therapeutic protein, e.g, a human anti-IL-12 antibody, or antigen-binding portion thereof, *e.g.*, an autoinjector pen or prefilled syringe comprises a dose of about 19 mg, 20, mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 31 mg, 32 mg, 33 mg, 34 mg, 35 mg, 36 mg, 37 mg, 38 mg, 39 mg, 40 mg, 41 mg, 42 mg, 43 mg, 44 mg, 45 mg, 46 mg, 47 mg, 48 mg, 49 mg, 50 mg, 51 mg, 52 mg, 53 mg, 54 mg, 55 mg, 56 mg, 57 mg, 58 mg, 59 mg, 60 mg, 61 mg, 62 mg, 63 mg, 64 mg, 65 mg, 66 mg, 67 mg, 68 mg, 69 mg, 70 mg, 71 mg, 72 mg, 73 mg, 74 mg, 75 mg, 76 mg, 77 mg, 78 mg, 79 mg, 80 mg, 81 mg, 82 mg, 83 mg, 84 mg, 85 mg, 86 mg, 87 mg, 88 mg, 89 mg, 90 mg, 91 mg, 92 mg, 93 mg, 94 mg, 95 mg, 96 mg, 97 mg, 98 mg, 99 mg, 100 mg, 101 mg, 102 mg, 103 mg, 104 mg, 105 mg, etc. of the formulation.

Non-therapeutic uses

The stable protein formulations comprising a protein at a concentration below the reverse stability concentration provided by the invention may also be used for non-therapeutic uses, *i.e., in vitro* purposes.

Stable protein formulations comprising a protein at a concentration below the reverse stability concentration described herein may be used for diagnostic or experimental methods

in medicine and biotechnology, including, but not limited to, use in genomics, proteomics, bioinformatics, cell culture, plant biology, and cell biology. For example, stable protein formulations comprising a protein at a concentration below the reverse stability concentration described herein may be used to provide a protein needed as a molecular probe in a labeling and detecting methods. An additional use for the formulations described herein is to provide supplements for cell culture reagents, including cell growth and protein production for manufacturing purposes.

Stable protein formulations comprising a protein at a concentration below the reverse stability concentration described herein could be used in protocols with reduced concern regarding how an excipient in the formulation may react with the experimental environment, *e.g.*, interfere with another reagent being used in the protocol. In another example, stable formulations containing protein concentrations below the reverse stability concentration may be used as a reagent for laboratory use.

Another alternative use for the formulation of the invention is to provide additives to food products. Thee formulation may be used to deliver high concentrations of a desired protein, such as a nutritional supplement, to a food item, without the presence of polyols, *e.g.*, sugar alcohols, which pass undigested into the large intestine. For example, whey- and soy-derived proteins are lending versatility to foods as these proteins have an ability to mimic fat's mouthfeel and texture. As such, whey- and soy-derived proteins may be added to foods to decrease the overall fat content, without sacrificing satisfaction. Thus, a stable protein formulation comprising suitable amounts of whey- and soy-derived proteins without polyols may be formulated and used to supplement food products.

Articles of Manufacture

In another embodiment of the invention, an article of manufacture is provided which contains the aqueous formulation of the present invention and provides instructions for its use. The article of manufacture comprises a container. Suitable containers include, for example, bottles, vials (e.g.,dual chamber vials), syringes (such as dual chamber syringes), autoinjector pen containing a syringe, and test tubes. The container may be formed from a variety of materials such as glass, plastic or polycarbonate. The container holds the aqueous formulation and the label on, or associated with, the container may indicate directions for use. For example, the label may indicate that the formulation is useful or intended for subcutaneous administration. The container holding the formulation may be a multi-use vial,

which allows for repeated administrations (e.g., from 2-6 administrations) of the aqueous formulation. The article of manufacture may further comprise a second container. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

The invention is further illustrated in the following examples, which should not be construed as further limiting.

EXAMPLES

The following examples provide experiments and experimental results aimed at improving the stability of pharmaceutical formulations of antibodies.

Materials and Methods

Size Exclusion HPLC Method

Size exclusion HPLC was used to determine the purity of the protein solution samples. The assay was performed with the parameters detailed below.

A TSK gel guard (cat. no. 08543, 6.0 mm x 4.0 cm, 7 μιη), was combined with a TSK gel G3000SW (cat. no. 08541, 7.8 mm x 30 cm, 5 μιη) and run with an upper column pressure limit of 70 bar. The mobile phase consisted of 100 mM Na_2HP0_4 / 200 mM Na_2S0_4, pH 7.0. This buffer was created by dissolving 49.68 g anhydrous disodium hydrogen phosphate and 99.44 g anhydrous sodium sulfate in approximately 3300 mL Milli-Q water, adjusting the pH to 7.0 using 1 M phosphoric acid, increasing the buffer volume to 3500 mL with Milli-Q water and filtering the solution through a membrane filter.

Experimental parameters were as follows:

- 0.3 ml/min flow rate
- *20* μL injection volume (equivalent to 20 μg sample)
- room temperature column
- 2 to 8°C autosampler temperature
- 50 minute run time
- isocratic gradient

Detection was done using a diode array detector using a 214 nm wavelength (> 0.1 min peak width and 8 nm band width) and a 360 nm reference wavelength (100 nm band width).

Test samples were run in duplicate. Purity was determined by comparing the area of the antibody peak to the total area of all 214 nm absorbing components in the sample, excluding buffer-related peaks. High molecular weight aggregates and antibody fragments can be resolved from intact the using this method.

Ultra Performance Liquid Chromatography (UPLC)

Ultra performance liquid chromatography (UPLC) was also used to determine the purity of the protein solution samples. Samples have a protein concentration of 1 mg/ml were prepared and analyzed to calibrate the retention and run times. The experimental parameters were as follows:

Mobile Phase: lOOmM Phosphate-200mM Sulfate, pH 6.8

Flow rate: 0.3ml/min

Detection: UV detection at 214nm and 280nm

Injection volumes: IOμI

Run time: 10 minutes per injection

Column: Waters ACQUITY UPLC BEH200 SEC 4.6 x 150 mm; 1.7μm, 200 Å.

Preparation of the antibody formulations for stability

The protein material used was:ABT-736: SUL 062808, ABT-874: SAP No. 10050811, Batch: 47006. The antibody stock formulations were prepared in the appropriate buffer solutions by applying DF/UF step. The solutions were sterile filtered through 0.22 micron Millipore syringe filters (Millipore, SLGV 013 SL) and filled in 0.5 mL vials (BioPlas, 4200S) under a laminar hood (Sterigard, BH6097RD). The sugar/polyols used in the study were: sucrose (J. T. Baker, 4074-05, Lot# H52479), Trehalose (Merck, K324961 16, Lot# 747), or Mannitol (Mallinckrodt, 6208-04, Lot# 48626).

Fluorescence Methods

Differential scanning fluorescence was used to measure the thermal stability of Briakinumab (=ABT-874) and ABT-736 in the absence and presence of sugars. The assay was performed with the parameters detailed below.

Briakinumab stock solutions were formulated in 10 mM Histidine, 10 mM Methionine, pH 6.0. The ABT-736 stock solutions were formulated in 15 mM Histidine, pH 6.0. Samples were prepared at 1, 100 and 200 mg/mL for all three antibodies containing 0, 25, 50, 75, 100, 125, 150, 175 or 200 mg/mL sugar/polyols. The sugar/polyols used in the study were: sucrose (J. T. Baker, 4074-05, Lot# H52479), sorbitol (Sigma-Aldrich, S-1876, Lot# 22H0026), Trehalose (Merck, K32496116, Lot# 747), or Mannitol (Mallinckrodt, 6208-04, Lot# 48626). Enough of a stock solution of SYPRO Orange (5000X concentration,

Invitrogen, S6650, Lot# 567391) was mixed with each sample to obtain a final concentration of SYPRO Orange of 0.4X.

For each condition 30 µl (n=3) of the sample solution was placed in a 96-well PCR microplate (Applied Biosystems, catalog # 3436906) and the plate was sealed with optical adhesive film (MicroAmp, catalog # NC9745301). The differential scanning fluorescence was run on a real-time PCR (Applied Biosystems, series 7500). For two minutes the samples were equilibrated at 25 °C, and then heated, in 1 °C intervals, from 25 to 95 °C. A heating rate of 4 °C/min was used to heat the samples for 15 seconds, and then the plate was equilibrated for one minute prior to measuring the emission from the samples using a TAMRA filter. From the thermograms the onset of melting was determined as the temperature at which the thermogram signal intensity was 3-fold higher than the baseline noise. The onset of melting of the antibodies was used as a measure of their thermal stability in the absence and presence of sugars/polyols.

Example 1: Impact of Sugars on ABT-736 Stability during Long-Term Storage (Table 2)

Fragmentation and aggregation are important degradation pathways influencing protein stability during long-term storage of liquid protein formulations. Especially aggregation is a degradation pathway that very often is observed in protein formulations, and the use of polyol excipients to increase protein stability in liquid protein formulations during shelf-life is a very established and accepted stabilization strategy formulators successfully apply-

To assess the stabilization potential of trehalose, a sugar routinely used in protein formulation to increase protein stability, ABT-736 samples were formulated at 200 mg/mL and 100 mg/mL in 15 mM histidine, pH 6 buffer, with (80 mg/mL trehalose) and without (0 mg/mL trehalose) trehalose, and all formulations were exposed to real-time stability studies (see Table 2A).

Storage stability under accelerated conditions at 40°C and 50°C was also evaluated to gain additional data (Table 2B and 2C) with sucrose formulations. Storage stability under accelerated conditions (50°C) for ABT-736 in higher ionic strength conditions (15mM Histidine + 100 mM NaCl buffer, pH 6) is provided in Table 2D. Real-time long-term stability (two months) of ABT-736 at 5oC was also determined for samples formulated in 15 mM histidine and higher ionic strength conditions (50 mM NaCl) with and without 80 mg/ml sucrose. The results of these studies are provided in Table 2E.

All of the data (as shown in Table 2 and in Figures 1 and 2) show that aggregation stability *(i.e.* physical stability of protein) can be dependent on protein concentration. At lower concentrations aggregation is increased by sugars/polyols (in concentrations of pharmaceutical interest) while at higher concentrations aggregation may be decreased. This result was very surprising, since this is exactly opposite to the crowding hypothesis and results in literature available today.

Samples were filled into 0.5 mL repositories and stored under controlled conditions (in temperature chambers and in the absence of light) at 5°C, 40°C and 50°C. At predefined points of time, samples of prepared solutions were pulled for analysis according to the sample pull scheme provided at the top of Table 1. Symbols refer to number of vials that were stored/pulled. Data is provided in Table 2.

Table 1: Sample pull schemes

for Table 2A:

Temperature(C)	T0	18m
5	XX	XX

for Tables 2B & 3A:

Temperature(C)	T0	3m
40	XX	XX

for Tables 2C & 3B:

Temperature(C)	T0	14d
50	XX	XX

for Tables 2D & 3C:

Temperature(C)	T0	9d
50	XX	XX

Table 2A: Impact of trehalose concentration on stability of ABT-736 at 5C (100 and 200 mg/mL, 15 mM Histidine buffer, pH 6). Average of duplicates.

200 mg/mL

	Trehalose concentration	

	0 mg/m L	80 mg/m L
Monomer		
TO	99.08	99.04
18m, 5C	93.21	92.98
Aggregate		
TO	0.91	0.95
18m, 5C	3.6	3.56
Fragment		
TO	0	0
18m, 5C	3.18	3.445

	100 mg/m L	
Monomer		
TO	99.08	99.04
18m, 5C	95.03	94.68
Aggregate		
TO	0.91	0.95
18m, 5C	1.79	1.87
Fragment		
TO	0	0
18m, 5C	3.16	3.47

Table 2B: Impact of sucrose concentration on stability of ABT-736 at 40C (15 mM Histidine buffer, pH 6).

Monomer					
		T0			
	No Sugar	3 %w/w	6 %w/w	9 %w/w	12 %w/w
Protein (mg/ml)					
198	91.8702	92.8302	92.4238	92.8331	92.9183
163	92.4051	92.6341	92.5475	92.3085	92.602
128	92.6236	92.1023	92.4779	92.7466	92.3737
93	92.777	92.8086	92.7222	92.5719	92.8546
58	92.794	92.2467	92.6795	92.744	92.6186
1	97.19	97.89	97.94	97.97	97.94
Aggregates					

198	3.9679	3.41 08	3.7597	3.3693	3.3877
163	3.3996	3.3591	3.441 2	3.6669	3.451 4
128	3.368	3.7021	3.4876	3.271 1	3.471
93	3.2461	3.2259	3.3598	3.371 4	3.3203
58	3.3681	3.681 5	3.288	3.2525	3.389
1	1.1	1.07	1.09	1.15	1.03
Fragments					
198	4.1 6 19	3.759	3.81 65	3.7977	3.694
163	4.1 953	4.0068	4.01 13	4.0246	3.9466
128	4.0084	4.1 956	4.0344	3.9822	4.1554
93	3.9769	3.9656	3.91 8	4.0567	3.825
58	3.8379	4.071 8	4.0325	4.0035	3.9923
1	1.69	0.94	0.95	0.86	1.01

		3m 40C			
Monomer					
	No Sugar	3 %w/w	6 %w/w	9 %w/w	12 %w/w
Protein (mg/ml)					
198	80.2	86.12	86.2	87.44	87.05
163	82.46	86.84	86.96	87.22	87.11
128	87.91	87.55	87.66	87.81	87.54
93	87.94	88.47	88.46	88.4	88.63
58	89.69	89.63	89.48	89.32	89.34
Aggregates					
198	9.55	7.18	7.31	5.9	6.03
163	7.9	6.47	6.34	6.13	6.16
128	5.22	5.68	5.58	5.51	5.64
93	4.7	4.6	4.61	4.79	4.66
58	3.46	3.65	3.69	3.75	3.77
Fragments					
198	9.84	6.69	6.48	6.64	6.91
163	9.62	6.67	6.68	6.64	6.71
128	6.85	6.76	6.75	6.67	6.81
93	7.35	6.91	6.82	6.79	6.7
58	6.84	6.71	6.82	6.91	6.87

Table 2C: Impact of sucrose concentration on stability of ABT-736 at 50C (14 days) (15 mM Histidine buffer, pH 6).

	50C, 14D				
	No Sugar	3 %w/w	6 %w/w	9 %w/w	12 %w/w
Monomer					
1mgml	92.38	91.99	91.96	91.87	91.94

Aggregates					
1mgml	1.8	2	1.92	2.1	1.86
Fragments					
1mgml	5.81	6	6.1	6.01	6.19
AUC					
1mgml	40374	41219	41983	40153	40485

	T0				
	No Sugar	3 %w/w	6 %w/w	9 %w/w	12 %w/w
Monomer					
Protein (mg/ml)	92.94	93.39	93.58	93.63	93.73
Aggregates					
Protein (mg/ml)	3.65	3.6	3.47	3.42	3.35
Fragments					
Protein (mg/ml)	3.39	3	2.94	2.93	2.9
AUC					
	35053	34486	32205	35774	40282

Table 2D: Impact of sucrose concentration on stability of ABT-736 at 50C (9 days) (15 mM Histidine + 100 Mm NaCl buffer, pH 6).

	50C, 9days				
	No Sugar	3 %w/w	6 %w/w	9 %w/w	12 %w/w
Protein (mg/ml)					
Monomer					
95	91.53	91.53	91.65	91.51	91.62
65	91.74	91.64	91.83	92.14	92.06
1	93.16	92.78		92.97	92.67
Aggregates					
95	3.9	3.9	3.86	3.79	3.69
65	3.33	3.77	3.42	3.24	3.25
1	2.4	2.55		2.46	2.53
Fragments					
95	4.56	4.5	4.48	4.69	4.68
65	4.92	4.57	4.74	4.61	4.68

1	4.44	4.66		4.55	4.79
AUC					
95	50644	5061 1	52690	46785	4 1729
65	32532	3701 5	34723	35473	34697
1	48843	43599		48648	4 1731

	T0				
	No Sugar	3 %w/w	6 %w/w	9 %w/w	12 %w/w
Protein (mg/ml)					
Monomer					
95	93.17	93.25	92.65	93.37	93.15
65	93.01	93.08	93.03	93.02	93.09
1	92.92	93.24	93.23	93.29	93.36
Aggregates					
95	3.78	3.76	4.05	3.7	3.8
65	3.9	3.86	3.83	3.85	3.82
1	4.06	3.8	3.83	3.76	3.72
Fragments					
95	3.03	2.97	3.28	2.91	3.03
65	3.07	3.04	3.12	3.12	3.07
1	3	2.95	2.93	2.93	2.9
AUC					
95	42450	43831	44244	53198	39903
65	34409	36606	33347	35309	31268
1	46636	45065	45748	48075	50893

Table 2E: Impact of sucrose concentration on stability of ABT-736 at 5°C (two months) (15 mM Histidine pH 6.0 and 15 mM Histidine + 50 Mm NaCl buffer, pH 6).

1 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	4.02	93.18	2.79
2 months, 5C	3.86	93.35	2.78
50 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	4.37	92.55	3.07
2 months, 5C	3.98	93.15	2.85
100 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments

T0	4.43	92.63	2.92
2 months, 5C	4.3	92.67	3.02
150 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	4.48	92.59	2.92
2 months, 5C	4.54	92.38	3.07
180 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	4.56	92.56	2.86
2 months, 5C	4.81	92.11	3.06
1 mg/mL	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	3.73	93.71	2.55
2 months, 5C	4.02	92.76	3.21
50 mg/ml	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	3.86	93.18	2.95
2 months, 5C	3.58	93.55	2.86
100 mg/ml	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	4.01	92.95	3.03
2 months, 5C	3.69	93.25	3.04
150 mg/ml	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	3.8	93.3	2.88
2 months, 5C	3.58	93.55	2.86
180 mg/ml	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	3.94	93.2	2.85
2 months, 5C	3.84	93.1	3.04
1 mg/mL	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	3.77	93.49	2.72
2 months, 5C	4.3	92.89	2.79
50 mg/ml	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	3.74	93.18	3.07

2 months, 5C	3.63	93.51	2.84
100 mg/ml	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	3.91	93.19	2.88
2 months, 5C	3.68	93.39	2.91
150 mg/ml	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	3.84	93.2	2.95
2 months, 5C	3.77	93.3	3.77
180 mg/ml	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	3.85	93.24	2.9
2 months, 5C	3.8	93.22	2.96
1 mg/mL	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	4.1	93.3	2.58
2 months, 5C	3.81	93.44	2.73
50 mg/ml	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	4.02	93.08	2.89
2 months, 5C	3.69	93.44	2.86
100 mg/ml	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	4.02	92.99	2.98
2 months, 5C	3.89	93.02	3.08
150 mg/ml	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	3.91	93.17	2.9
2 months, 5C	3.92	93.22	2.84
180 mg/ml	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	4.04	93.01	2.93
2 months, 5C	3.84	93.28	2.86

ABT-736 stability decreased over time at 5°C conditions, and both aggregation and fragmentation was observed. The sugars had a destabilizing influence at 100 mg/mL. It is evident from this experiment that the addition of trehalose and sucrose to lower concentration ABT-736 liquid formulations *(e.g.,* at 100 mg/mL protein) results in ABT-736 formulations with decreased stability, *i.e.,* levels of aggregate are higher and levels of monomer are lower in ABT-736 formulations that contain trehalose than in formulations without trehalose. This was highly surprising, given the stabilization potential of sugars such as trehalose reported in literature describing protein formulations, including commercial antibody formulations such as Avastin, Herceptin, or Lucentis. Also, the data reported in Example 3 *(see* Table 4) clearly suggests that the intrinsic stability of ABT-736 in pH 6, 15 mM histidine buffer formulation is higher, the more sucrose is present in the formulation. According to the current understanding of DSC/DSF experiments and how to apply those data in a protein formulation laboratory, higher intrinsic stability of ABT-736 as determined in characterization studies such as DSF or DSC should be reflected in higher stability and decreased aggregate levels during accelerated stability studies. Thus, these results of ABT-736 liquid formulation stability studies were surprising.

Example 2: Impact of Sucrose Concentration on ABT-874 Stability during Accelerated And Long-Term Storage (Table 3)

To further assess the stabilization potential of sucrose, a sugar routinely used in protein formulations to increase protein stability, additional experiments were carried out with the antibody ABT-874. ABT-874 samples were formulated with and without sucrose, and all formulations were exposed to real-time stability studies (see Table 3D). In addition, ABT-874 formulations were subjected to short-term storage at elevated temperatures (40°C) in order to relatively quickly gain insight in the sucrose stabilization potential for long-term storage at lower temperatures (often at 2-8°C) (Table 3A). Storage stability under accelerated conditions at 50°C was also evaluated to gain data additional (Table 3B). Storage stability under accelerated conditions (50°C) for ABT-874 in higher ionic strength conditions (15mM Histidine + 100 mM NaCl buffer, pH 6) is provided in Table 3C.

The data that have also been summarized in Figure 2 show that aggregation stability *(i.e.,* physical stability of protein) may be dependent on protein concentration. At lower

concentrations of the protein aggregation is increased by sugars/polyols (in concentrations of pharmaceutical interest) while at higher concentrations of the protein *(e.g.,* in low ionic formulations) aggregation is decreased. This is exactly opposite to results reported in the literature.

Samples were filled into 0.5 mL repositories and stored under controlled conditions (in temperature chambers and in the absence of light) at 40°C and 50°C. At predefined points of time, samples of prepared solutions were pulled for analysis according to the sample pull scheme provided in Table 1 (above). Numbers refer to number of vials that were stored/pulled. Data is provided in Table 3A, 3B and 3C.

Table 3A. ABT-874, Impact of sucrose. 40°C data in 15mM Histidine at pH 6.

	No Sugar	3 %w/w	6 %w/w	9 %w/w	12 %w/w
Protein (mg/ml)					
	T0				
Monomer					
185	97.0502	97.0653	97.0248	97.248	97.365
155	97.0907	96.9593	97.0461	97.143	97.088
125	96.9336	97.1624	97.1745	96.7804	96.9932
95	96.4007	97.2211	96.986	97.1747	97.1601
65	97.0998	97.2914	97.3085	97.3488	97.3174
1	94.09	92.93	92.45	92.75	92.78
Aggregates					
185	1.2899	1.1328	1.2408	0.9585	0.8663
155	1.194	1.2793	1.1265	1.0046	1.1527
125	1.299	1.0958	1.0047	1.2403	1.0709
95	1.7564	0.9857	1.093	1.1624	1.0431
65	1.1172	0.889	0.8982	0.8706	0.954
1	2.57	4.18	4.43	4.25	4.32
Fragments					
185	1.6599	1.8019	1.7344	1.7935	1.7688
155	1.7152	1.7614	1.8094	1.8524	1.7585
125	1.7674	1.7418	1.8208	1.9794	1.9359
95	1.8429	1.7932	1.921	1.6629	1.7968
65	1.783	1.8196	1.7934	1.7806	1.7286
1	3.32	2.88	3.1	2.99	2.89
		3m 40C			
Monomer					
185	90.06	90.45	90.12	90.56	90.48
155	89.66	90.74	90.88	90.69	90.84

125	90.75	91.21	91.16	91.24	90.87
95	90.89	91.55	91.78	91.57	90.95
65	91.52	92.09	92.37	91.7	91.1
Aggregates					
185	4.13	3.58	3.7	3.16	2.93
155	4.19	3.45	3.26	3.36	3.27
125	3.34	3.03	3.01	2.93	3.31
95	3.04	2.68	2.56	2.64	3.25
65	2.62	2.23	2.14	2.55	3.03
Fragments					
185	5.8	5.95	6.17	6.27	6.58
155	6.14	5.8	5.85	5.93	5.87
125	5.89	5.74	5.81	5.82	5.8
95	6.05	5.75	5.65	5.78	5.79
65	5.85	5.67	5.47	5.73	5.86

Table 3B. ABT-874, Impact of sucrose. 50°C (14 days) data in 15mM Histidine at pH 6.

	50C, 14D				
	No Sugar	3 %w/w	6 %w/w	9 %w/w	12 %w/w
Protein (mg/ml)					
Monomer					
1mg/ml	94.09	93.76	93.82	93.67	93.89
Aggregates					
1mg/ml	1.17	1.12	1.06	1.16	1.16
Fragments					
1mg/ml	4.73	5.11	5.1	5.16	5.06
AUC					
	35305	35398	36082	35917	35858

	T0				
	No Sugar	3 %w/w	6 %w/w	9 %w/w	12 %w/w
Monomer					
Protein (mg/ml)	97.67	97.61	97.62	97.63	97.68
Aggregates					
Protein (mg/ml)	0.81	0.87	0.87	0.87	0.89
Fragment					
Protein (mg/ml)	1.5	1.51	1.5	1.49	1.41

AUC					
	30849	31309	30580	31525	33338

Table 3C: Impact of Sucrose Concentration on Stability of ABT-874 at 50°C (9 days) (15 mM Histidine + 100 Mm NaCl buffer, pH 6).

	No Sugar	3 %w/w	6 %w/w	9 %w/w	12 %w/w
Protein (mg/ml)					
	50℃ 9d				
Monomer					
95	94.02	94.13	94.28	94.18	94.3
65	94.45	94.66	94.69	94.55	
1	95.92	96	96.06	96.06	95.99
Aggregates					
95	2.97	3	2.91	2.98	2.86
65	2.61	2.48	2.43	2.54	
1	1.43	1.39	1.4	1.34	1.24
Fragments					
95	2.99	2.84	2.79	2.8	2.83
65	2.93	2.85	2.86	2.89	
1	2.63	2.59	2.53	2.58	2.76
AUC					
95	38444	38819	37518	38544	35941
65	28979	28123	29062	30751	
1	67538	68981	74487	77386	74915
	T0				
Monomer					
95	97.46	97.49	97.41	97.46	97.43
65	97.47	97.47	97.48	97.56	97.54
1	97.97	97.94	97.98	97.87	97.94
Aggregates					
95	1.6	1.6	1.58	1.59	1.59
65	1.48	1.5	1.49	1.38	
1	1.17	1.23	1.28	1.34	1.29
Fragments					
95	0.92	0.9	0.98	0.94	0.96
65	1.03	1.02	1.02	1.05	1.04
1	0.84	0.82	0.72	0.78	0.76
AUC					

95	51365	58524	43594	44215	39814
65	28545	26833	29304	26776	26829
1	64135	76658	72903	87616	74460

The results at 40°C as shown in Tables 3A show the same destabilizing effect of polyols on low ionic strength protein formulations as observed for ABT-736.

Table 3D. ABT-874, Impact of sucrose at 5°C data in 15mM Histidine at pH 6 and in 15mM Histidine + 50 mM NaCl at pH 6.

1 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	0.89	98.26	0.83
2 months, 5C	0.84	98.24	0.91
50 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	1.17	97.81	1.01
2 months, 5C	1.17	97.81	1
100 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	1.29	97.65	1.04
2 months, 5C	1.45	97.4	1.13
150 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	1.4	97.52	1.06
2 months, 5C	1.38	97.52	1.09
180 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	1.5	97.43	1.06
2 months, 5C	1.26	97.67	1.06
1mg/mL	15 mM Histidine + 50 mM NaCl	Monomer	Fragments
	Aggregates		
T0	0.96	98.19	0.84
2 months, 5C	0.69	98.45	0.84
50 mg/ml	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	1.31	97.74	0.93
2 months, 5C	0.99	98.04	0.96
100 mg/ml	15 mM Histidine + 50 mM NaCl		

	Aggregates	Monomer	Fragments
T0	1.36	97.6	1.03
2 months, 5C	1.2	97.74	1.04
150 mg/ml	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	1.63	97.33	1.02
2 months, 5C	1.43	97.54	1.02
180 mg/ml	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	1.4	97.6	0.99
2 months, 5C	1.43	97.55	1
1 mg/mL	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	0.86	98.07	1.05
2 months, 5C	0.57	98.48	0.94
50 mg/ml	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	0.7	98.39	0.9
2 months, 5C	0.67	98.31	1.01
100 mg/ml	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	0.68	98.32	0.98
2 months, 5C	0.59	98.41	0.99
150 mg/ml	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	0.68	98.42	0.89
2 months, 5C	0.59	98.44	0.95
180 mg/ml	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	0.67	98.5	0.81
2 months, 5C	0.66	98.3	1.03
1 mg/mL	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	0.72	98.4	0.86
2 months, 5C	0.65	98.46	0.87

50 mg/ml	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	1.19	97.76	1.03
2 months, 5C	1.12	97.91	0.95
100 mg/ml	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	1.08	97.96	0.94
2 months, 5C	0.96	98.17	0.85
150 mg/ml	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	1.24	97.74	1
2 months, 5C	1.12	97.9	0.97
180 mg/ml	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	1.26	97.8	0.93
2 months, 5C	0.98	98	1

Example 3: Impact of Concentration of Sugars on ABT-736 and 874 Intrinsic Stability (Thermodynamic Stability)

Differential scanning fluorescence was used to measure the thermal stability of ABT-736 and 874 in the absence and presence of sugars. The assay was performed with the parameters detailed in the method section. As shown in Table 4, an increase in polyol concentration lead to a progressive increase in intrinsic stability/unfolding temperature of ABT-736 and 874 at all concentrations. This was found to be true for all sugars tested *i.e.* sucrose, trehalose, mannitol and sorbitol. The sugar concentration was varied from 0 to 120 mg/ml except for mannitol wherein the solubility restricted us to 150 mg/mL. This increase in the unfolding temperature (that is, an increase in intrinisic or thermodynamic stability) is expected to reflect in increased physical stability of liquid formulations. This is due to the fact that proteins are believed to aggregate upon unfolding and since sugars prevent the unfolding of the proteins, they are anticipated to decrease the aggregation and hence increase the physical stability of the macromolecules (Chi, Eva Y.; Krishnan, Sampathkumar; Kendrick,

Brent S.; Chang, Byeong S.; Carpenter, John F.; Randolph, Theodore W. Roles of conformational stability and colloidal stability in the aggregation of recombinant human granulocyte colony-stimulating factor. Protein Science (2003), 12(5), 903-913). Surprisingly, the opposite effect was observed, *i.e.,* the presence of sugars and sugar alcohols in liquid protein solutions decreased the physical stability (see Example 1) when stability was assessed for long-term storage (18m, 5C) of ABT-736 at 100 mg/mL (see Figure 1A).

Table 4. Thermal Stability of ABT-736 and 874 Antibodies in the Presence of Sucrose as Determined by Differential Scanning Fluorescence

	736(mg/ml)					
	198	163	128	93	58	1
Sucrose (%)						
0	53.25	54	54.75	54.75	55.25	60.5
3	54.75	54.75	55	55.5	56	61.5
6	54	55	55.5	55.5	55.5	62
9	54	55.25	55.75	55.5	56.25	62.5
12	55.5	55.75	55.75	56	56.5	62.4
	185	155	125	85	65	1
	874 (mg/ml)					
	185	155	125	95	55	1
0	53.25	55	54.5	55.25	56.75	60.3
3	53.25	53.75	54.5	55.25	56.75	62.5
6	54.5	54.25	56.25	56.25	57	62.7
9	55.25	54	56.25	57	57.75	62.5
12	56	55.75	56.75	57.25	58	63.1

Example 4: Impact of Sugars on Anti-HER2 Antibody Stability during Long-Term Storage

To further assess the stabilization potential of sucrose, a sugar routinely used in protein formulations to increase protein stability, additional experiments were carried out with a humanized IgGl kappa monoclonal antibody that selectively binds with high affinity to the extracellular domain of the human epidermal growth factor receptor 2 protein (HER2). Samples comprising the anti-HER2 antibody were formulated with and without sucrose and with and without NaCl, and all formulations were exposed to real-time stability studies (see Table 5).

The data show that aggregation stability *{i.e.,* physical stability of the protein) is dependent on protein concentration. At lower concentrations of the antibody aggregation is increased by sugars/polyols (in concentrations of pharmaceutical interest) while at higher

concentrations of the antibody *(e.g.,* in low ionic formulations) aggregation is decreased by sugars/polyols (in concentrations of pharmaceutical interest). This is exactly opposite to results reported in the literature.

Table 5. Impact of sucrose on anti-HER2 antibody in 15mM Histidine at pH 6 and in 15mM Histidine + 50 mM NaCl at pH 6.

1 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	3.13	96.6	0.27
3 months, 5C	0.76	98.96	0.27
50 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	0.9	98.75	0.33
3 months, 5C	0.78	98.93	0.28
100 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	1.41	98.31	0.29
3 months, 5C	1.4	98.29	0.31
150 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	1.72	98.04	0.24
3 months, 5C	1.72	97.92	0.35
180 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	1.73	98.02	0.25
3 months, 5C	1.82	97.87	0.31
1 mg/mL	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	0.88	98.89	0.22
3 months, 5C	0.79	98.98	0.24
50 mg/ml	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	1.09	98.44	0.45
3 months, 5C	0.89	98.86	0.26
100 mg/ml	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	1.25	98.43	0.33
3 months, 5C	1.23	98.51	0.26

150 mg/ml	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	1.52	98.15	0.34
3 months, 5C	1.48	98.25	0.27
180 mg/ml	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	1.56	98.09	0.36
3 months, 5C	1.44	98.3	0.26
1 mg/mL	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	0.9	98.87	0.24
3 months, 5C	0.56	99.23	0.21
50 mg/ml	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	0.52	99.13	0.35
3 months, 5C	0.54	99.12	0.34
100 mg/ml	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	0.86	98.91	0.23
3 months, 5C	0.87	98.89	0.25
150 mg/ml	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	1.03	98.75	0.22
3 months, 5C	1.02	98.71	0.27
180 mg/ml	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	1	98.75	0.25
3 months, 5C	1.05	98.71	0.23
1 mg/mL	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	0.71	99.1	0.19
3 months, 5C	0.65	99.17	0.19
50 mg/ml	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments

T0	0.63	99.05	0.32
3 months, 5C	0.69	99.02	0.3
100 mg/ml	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	0.93	98.83	0.24
3 months, 5C	1.02	98.72	0.26
150 mg/ml	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	1.2	98.49	0.31
3 months, 5C	1.12	98.62	0.25
180 mg/ml	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	1.1	98.57	0.32
3 months, 5C	1.09	98.66	0.25

Example 5: Impact of Sugars on an anti-VEGF Antibody Stability during Long-Term Storage

To further assess the stabilization potential of sucrose, a sugar routinely used in protein formulations to increase protein stability, additional experiments were carried out with a humanized IgGl kappa monoclonal antibody that binds to and inhibits the biologic activity of human vascular endothelial growth factor (VEGF).

Antibody samples were formulated with and without sucrose and with and without NaCl, and all formulations were exposed to real-time stability studies (see Table 6). The data show that aggregation stability *(i.e.,* physical stability of the protein) is dependent on protein concentration. At lower concentrations of the antibody aggregation is increased by sugars/polyols (in concentrations of pharmaceutical interest) while at higher concentrations of the antibody *(e.g.,* in low ionic formulations) aggregation is decreased by sugars/polyols (in concentrations of pharmaceutical interest). This is exactly opposite to results reported in the literature.

Table 6. Impact of sucrose on anti-VEGF antibody in 15mM Histidine at pH 6 and in 15mM Histidine + 50 mM NaCl at pH 6.

1 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	0.64	99.1	0.26
3 months, 5C	3.63	96.02	0.36
50 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	4.22	95.17	0.61
3 months, 5C	4.68	94.85	0.47
100 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	8.97	90.62	0.41
3 months, 5C	6.94	92.65	0.41
150 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	11.14	88.51	0.35
3 months, 5C	7.96	91.68	0.36
180 mg/ml	15 mM Histidine		
	Aggregates	Monomer	Fragments
T0	10.98	88.71	0.32
3 months, 5C	8.22	91.33	0.45
1 mg/mL	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	3.9	95.8	0.3
3 months, 5C	3.95	95.73	0.32
50 mg/ml	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	4.12	95.34	0.54
3 months, 5C	4.03	95.49	0.48
100 mg/ml	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	6.47	93.15	0.38
3 months, 5C	5.28	94.29	0.42
150 mg/ml	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	7.55	92.12	0.33

3 months, 5C	5.87	93.69	0.44
180 mg/ml	15 mM Histidine + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	6.89	92.78	0.33
3 months, 5C	5.87	93.67	0.46
1 mg/mL	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	4.11	95.55	0.34
3 months, 5C	3.03	96.7	0.27
50 mg/ml	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	3.46	96.01	0.53
3 months, 5C	3.8	95.76	0.45
100 mg/ml	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	4.48	94.84	0.31
3 months, 5C	4.6	95.04	0.36
150 mg/ml	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	5.04	94.62	0.33
3 months, 5C	4.8	94.85	0.35
180 mg/ml	15 mM Histidine + 80 mg/mL sucrose		
	Aggregates	Monomer	Fragments
T0	5.13	94.53	0.34
3 months, 5C	4.84	94.71	0.46
1 mg/mL	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	3.04	96.69	0.27
3 months, 5C	3.29	96.43	0.28
50 mg/ml	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	3.27	96.24	0.48
3 months, 5C	3.33	96.3	0.37
100 mg/ml	15 mM Histidine + 80 mg/mL sucrose + 50 mM		

	NaCl		
	Aggregates	Monomer	Fragments
T0	4.18	95.46	0.36
3 months, 5C	4.02	95.66	0.33
150 mg/ml	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	4.29	95.33	0.38
3 months, 5C	4.34	95.28	0.39
180 mg/ml	15 mM Histidine + 80 mg/mL sucrose + 50 mM NaCl		
	Aggregates	Monomer	Fragments
T0	4.47	95.1	0.43
3 months, 5C	4.18	95.4	0.42

Example 6: Determining Reverse Stability Concentrations

The data presented herein indicate that polyols *(e.g.,* sugars) act in some situations as crowders and can decrease the colloidal stability of the molecules by forcing protein molecules into closer proximity under certain formulation conditions *(e.g.,* this can impact the second step of the aggregation process) (see Figure 3). This perturbation results in the destabilization effect that is surprising. The destabilization of lower protein concentration formulations by polyols is a new, surprising finding.

Conformational (thermal) and stability studies may be conducted for various concentrations of polyols and/or proteins for various periods of time. For example, in one set of experiments, formulations comprising about 1-200 mg/mL concentrations of protein *(e.g.,* ABT-736 and 874) in combination with 0, 30, 60, 90, 120 mg/mL concentrations of sucrose are used in conformational and stability studies. These different formulations are subjected to stability studies at various temperatures *(e.g.,* 5, 25 and 40°C). The formulations are also investigated for thermal stability using dynamic scanning fluorescence in order to determine the stabilization potential of the sugars. Size exclusion chromatography is used to monitor protein aggregation in the formulations.

For each fixed concentration of polyol that undergoes confirmational and stability studies, a limit to the amount of protein that may be contained by the formulation without loss of the stabilizing effects of the polyol at that concentration is determined. Size exclusion

chromatography is used as primary analytical method to monitor protein aggregation throughout these experiments.

INCORPORATION BY REFERENCE

The contents of all cited references (including, for example, literature references, patents, patent applications, and websites) that maybe cited throughout this application are hereby expressly incorporated by reference in their entirety for any purpose. The practice of the present invention will employ, unless otherwise indicated, conventional techniques of protein formulations, which are well known in the art.

EQUIVALENTS

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting of the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are therefore intended to be embraced herein.

What is claimed:

1. A protein formulation having enhanced shelf-life stability relative to a first protein formulation, wherein the first protein formulation comprises a polyol at a first polyol concentration, the protein formulation comprising

a protein at a concentration below a Reverse Stability Concentration for the protein in the first protein formulation; and

no polyol or a polyol at a concentration below the first polyol concentration.

2. The protein formulation of claim 1, wherein the protein concentration in the protein formulation is greater than the protein concentration in the first protein formulation

3. The protein formulation of claim 1, wherein the formulation is substantially free of protein aggregation in a liquid form at 5°C for a period of time selected from the group consisting of at least 3 months, at least 6 months, at least 12 months, at least 18 months and at least 24 months.

4. The protein formulation of claim 1, wherein the formulation comprises protein aggregation at a level selected from the group consisting of less than 10% protein aggregation, less than 5% protein aggregation, less than 2% protein aggregation and less than 1% aggregation following storage at room temperature for at least 3 months.

5. The protein formulation of claim 1, wherein the polyol concentration is selected from the group consisting of below about 200 mg/ml, below about 120 mg/ml, below about 80 mg/ml and below about 40 mg/ml.

6. The protein formulation of claim 1, wherein the Reverse Stability Concentration of the protein is between about 100 mg/mL and 150 mg/mL.

7. The protein formulation of claim 1, wherein the Reverse Stability Concentration of the protein is between about 125 mg/mL and 135 mg/mL.

8. The protein formulation of claim 1, wherein the protein concentration is selected from the group consisting of at least about 25 mg/mL, at least about 50 mg/ml, at least about 100 mg/ml and at least about 150 mg.

9. The protein formulation of claim 1, wherein the protein formulation comprises one or more distinct proteins, wherein at least one of the proteins is a therapeutic protein.

10. The protein formulation of claim 1, wherein the protein is an antibody, or an antigen-binding portion thereof.

11. The protein formulation of claim 1, wherein the antibody, or an antigen-binding portion thereof, comprises a lambda light chain or a kappa light chain

12. The protein formulation of claim 1, wherein the polyol is selected from the group consisting of sucrose, trehalose, mannitol and sorbitol.

13. A method of treating a disorder in a subject, comprising administering to the subject the formulation of any one of claims 1-9.

14. A method of determining the Reverse Stability Concentration of a protein in a formulation comprising a polyol, the method comprising:

preparing a series of formulations comprising a protein and a polyol, wherein the protein concentration in the formulations is constant and the polyol concentration is varied over a range of concentrations, and wherein the concentration of other solutes in the formulations is the same;

exposing the series of formulations to a test condition;

measuring the level of protein aggregation in the series of formulations after exposure to the test condition; and

determining the presence of a change in the level of aggregation in the series of formulations over the range of polyol concentration,

wherein an absence of a change in the level of aggregation in the series of formulations over the range of polyol concentrations indicates that the protein concentration is the Reverse Stability Concentration of the protein in the formulation.

15. A method of detecting the Reverse Stability Concentration of a protein in a formulation comprising a polyol, the method comprising:

preparing a first series of formulations comprising a protein at a first protein concentration and a polyol, wherein the polyol concentration is varied over a range;

preparing a second series of formulations comprising a protein at a second protein concentration that is higher than the first protein concentration and a polyol, wherein the polyol concentration is varied over a range, and wherein the concentration of other solutes in the first series of formulations and second series of formulations is the same;

exposing the first series of formulations and the second series of formulations to a test condition;

measuring the level of protein aggregation in the first series of formulations and the second series of formulations after exposure to the test condition; and

determining the change in the level of aggregation in each series of formulations from lowest to highest polyol concentration,

wherein an increase in the level of aggregation in the first series of formulations and a decrease in the level of aggregation in the second series indicates that the Reverse Stability Concentration is between the first protein concentration and the second protein concentration for the protein in the formulation comprising a polyol.

16. A method of detecting the existence of a Reverse Stability Concentration of a protein in a formulation comprising a polyol at a given ionic strength, comprising:

preparing a first formulation and a second formulation, wherein the concentration of polyol is lower in the first formulation than in the second formulation, and wherein the concentration of the protein in the first formulation and second formulation is the same;

exposing the first and second formulations to a test condition; and

measuring protein aggregation in the first and second formulations after testing,

wherein greater protein aggregation in the second formulation as compared to the first formulation indicates that a Reverse Stability Concentration exists for the protein in the formulation comprising the polyol.

17. The method of any one of claims 14-16, wherein the test condition comprises storage at a temperature selected from the group consisting of 5°C, 40°C and 50°C.

18. The method of any one of claims 14-16, wherein the test condition comprises storage for a period of time selected from the group consisting of at least about 3 months, at least 6 months, at least 12 months, at least 18 months and at least 24 months.

19. The method of any one of claims 14-16, wherein protein aggregation is measured by size exclusion HPLC or nephelometry.

20. The method of any one of claims 14-16, wherein the protein is a therapeutic protein.

21. The method of any one of claims 14-16, wherein the protein is an antibody, or an antigen-binding portion thereof.

22. The method of claim 21, wherein the antibody, or an antigen-binding portion thereof, comprises a lambda light chain or a kappa light chain.

23. The method of any one of claims 14-16, wherein the protein concentration of the formulations is selected from the group consisting of below about 125 mg/mL, below about 100 mg/ml, below about 80 mg/ml, below about 60 mg/ml and below about 40 mg/ml.

24. The method of any one of claims 14-16, wherein the polyol is selected from the group consisting of trehalose, mannitol, sucrose and sorbitol.

25. A method for reducing protein aggregation in a formulation comprising a protein and a polyol, the method comprising:

determining a Reverse Stability Concentration for the protein in the formulation, and

reducing the concentration of the polyol in the formulation if the protein concentration is lower than the Reverse Stability Concentration,

thereby reducing protein aggregation in the formulation.

26. A method for reducing protein aggregation in a formulation comprising a protein and a polyol, the method comprising:

selecting a formulation comprising a protein and a polyol;

identifying the protein concentration in the formulation as above or below the Reverse Stability Concentration for the protein in the formulation; and

reducing the concentration of the polyol when the protein concentration is below the Reverse Stability Concentration for the protein in the formulation,

thereby reducing protein aggregation in the formulation.

27. A method for preparing a stable formulation having increased protein concentration as compared to a first formulation, the method comprising:

selecting a first formulation comprising a protein at a first protein concentration and a polyol at a first polyol concentration;

determining a Reverse Stability Concentration for the protein in the first formulation;

increasing the protein concentration in the formulation to a concentration that is above the first protein concentration and below the Reverse Stability Concentration; and

reducing the concentration of the polyol in the formulation below the first polyol concentration,

thereby preparing a stable formulation having increased protein concentration as compared to the first formulation.

28. The method of any one of claims 25-27, wherein the polyol concentration is reduced to less than about 200 mg/ml, less than about 120 mg/ml, less than about 80 mg/ml and less than about 40 mg/ml.

29. The method of any one of claims 25-27, wherein the polyol concentration is reduced to 0.

30. The method of any one of claims 25-27, wherein the protein formulation is substantially free of protein aggregation in a liquid form at 5°C for a period of time selected from the group consisting of at least 3 months, at least 6 months, at least 12 months, at least 18 months and at least 24 months.

31. The method of any one of claims 25-27, wherein the protein formulation comprises protein aggregation at a level selected from the group consisting of less than 10% protein

aggregation, less than 5% protein aggregation, less than 2% protein aggregation and less than 1% aggregation following storage at room temperature for at least 3 months.

5

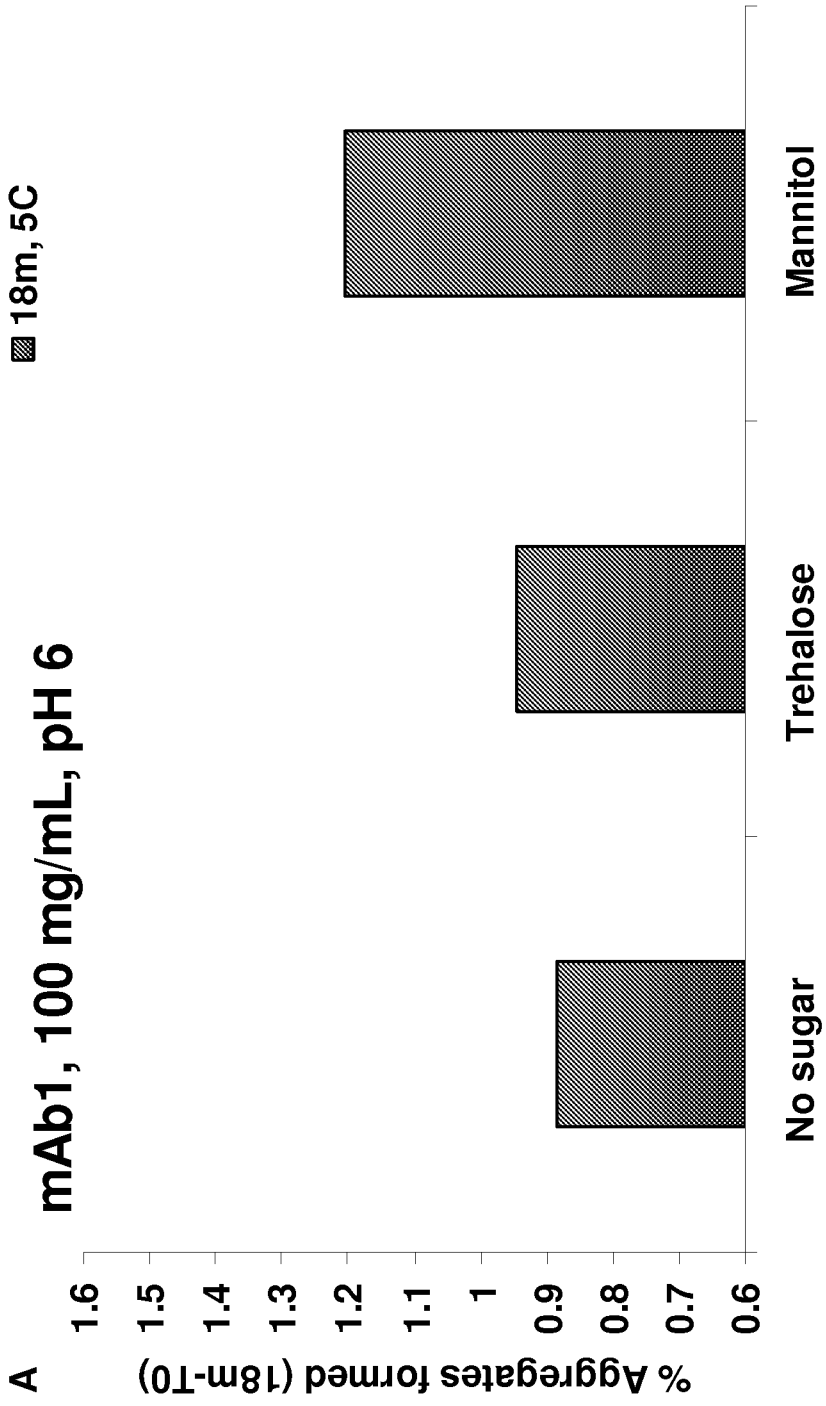
A
% Aggregates formed (18m-T0)
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
mAb1, 100 mg/mL, pH 6
18m, 5C
No sugar
Trehalose
Mannitol

Figure 1A

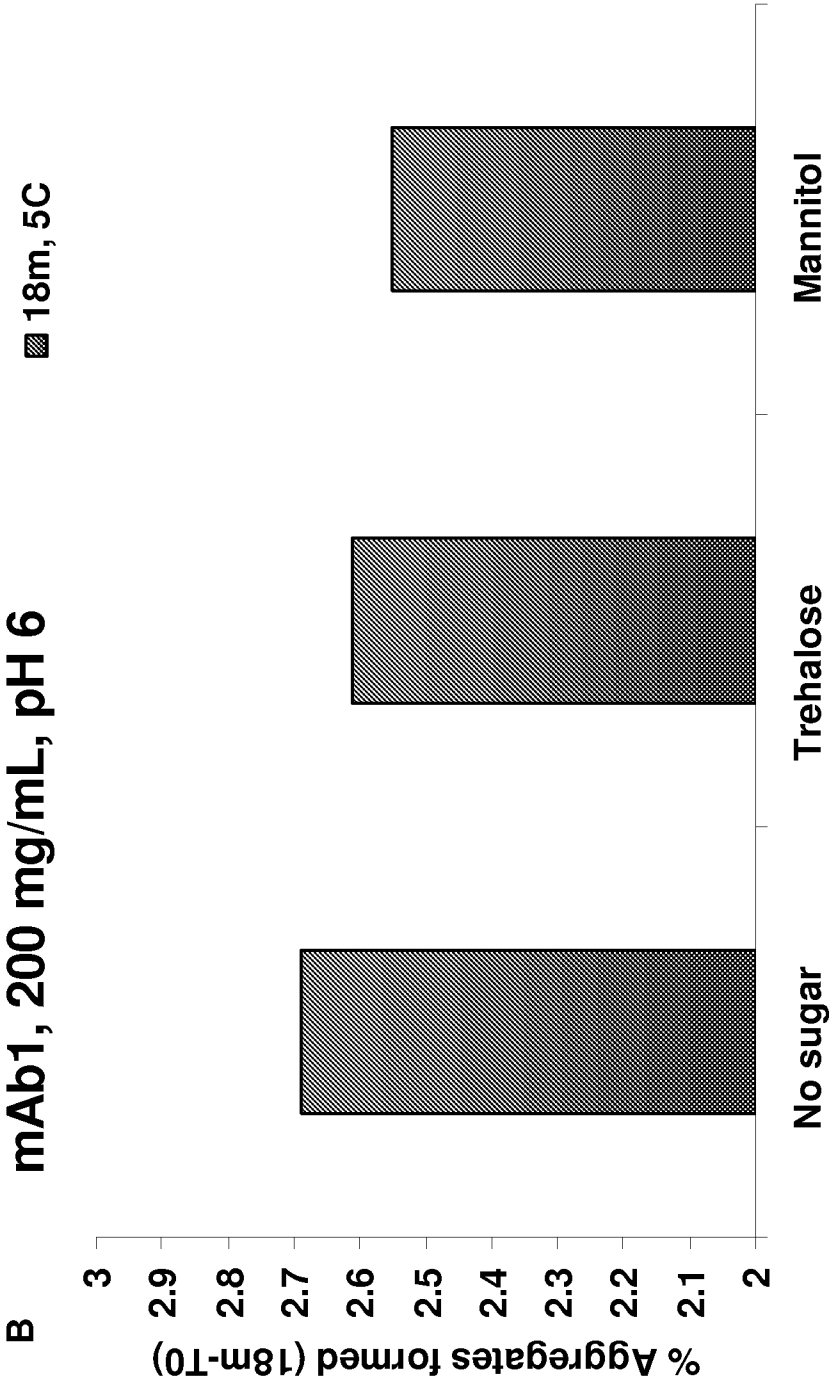
B
mAb1, 200 mg/mL, pH 6
18m, 5C
% Aggregates formed (18m-T0)
3
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2
No sugar
Trehalose
Mannitol

Figure 1B

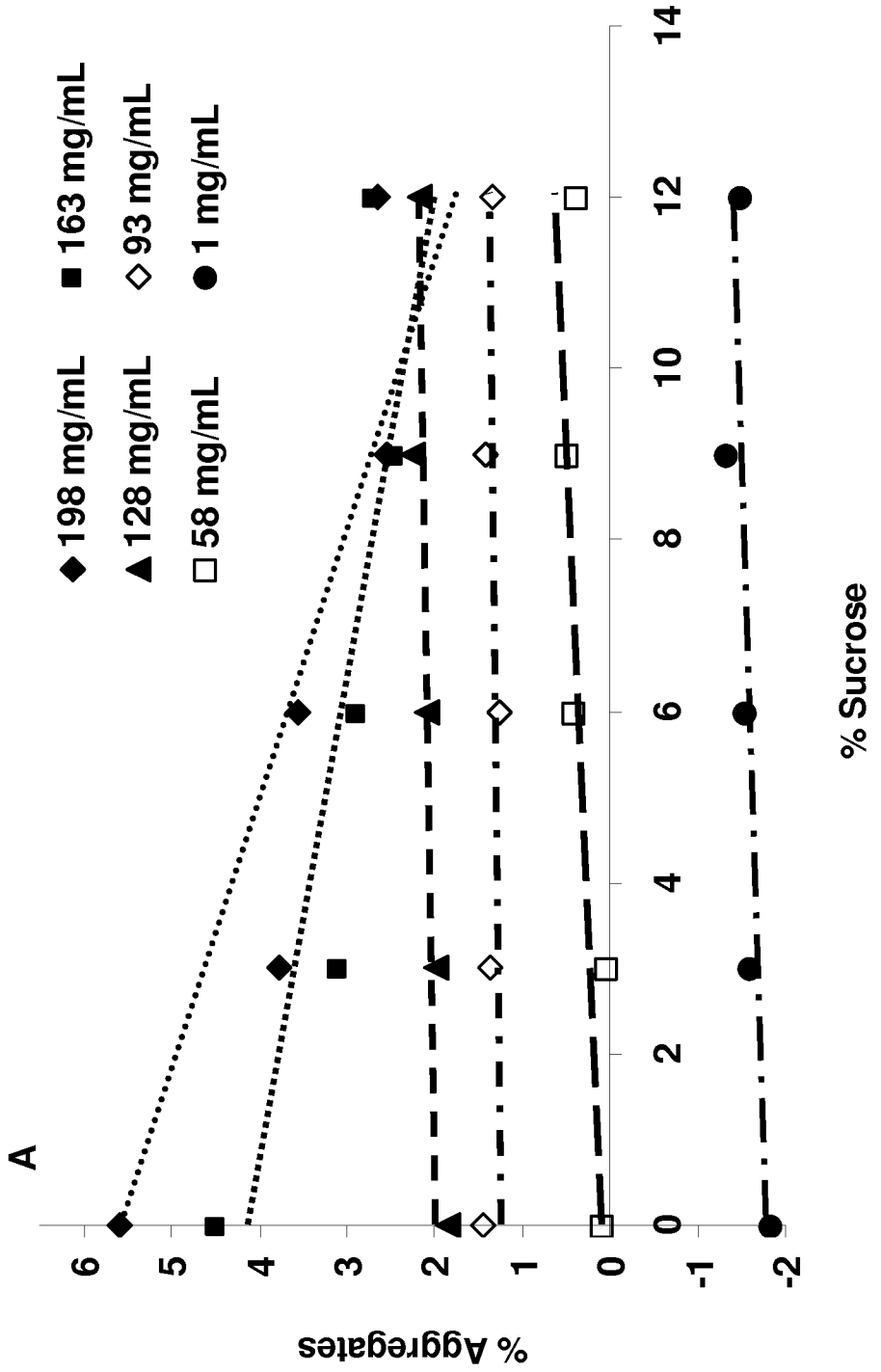
A
198 mg/mL
163 mg/mL
128 mg/mL
93 mg/mL
58 mg/mL
1 mg/mL
% Aggregates
% Sucrose
6
5
4
3
2
1
0
-1
-2
0
2
4
6
8
10
12
14

Figure 2A

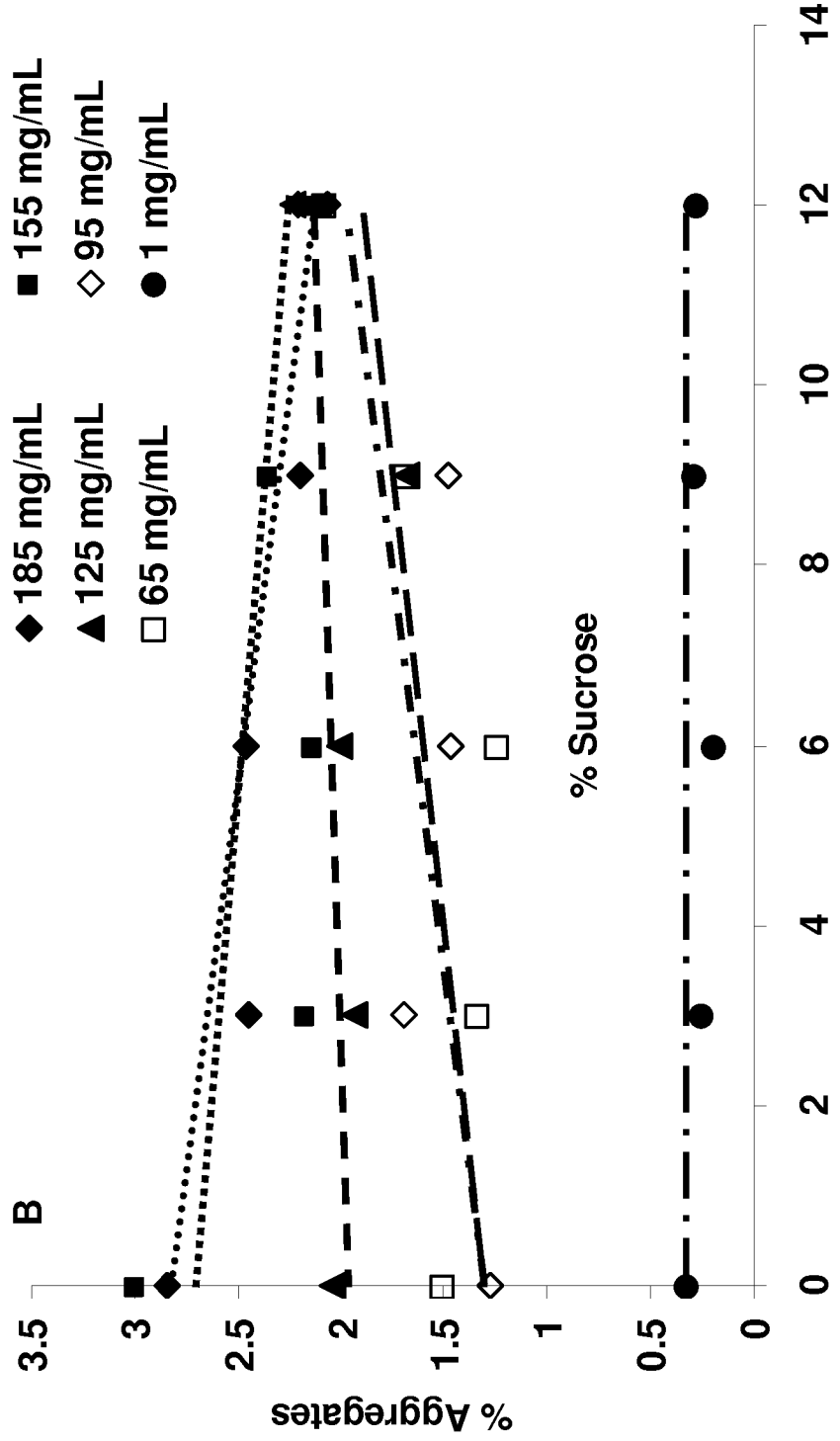
B
185 mg/mL
155 mg/mL
125 mg/mL
95 mg/mL
65 mg/mL
1 mg/mL
% Aggregates
% Sucrose
3.5
3
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12
14

Figure 2B

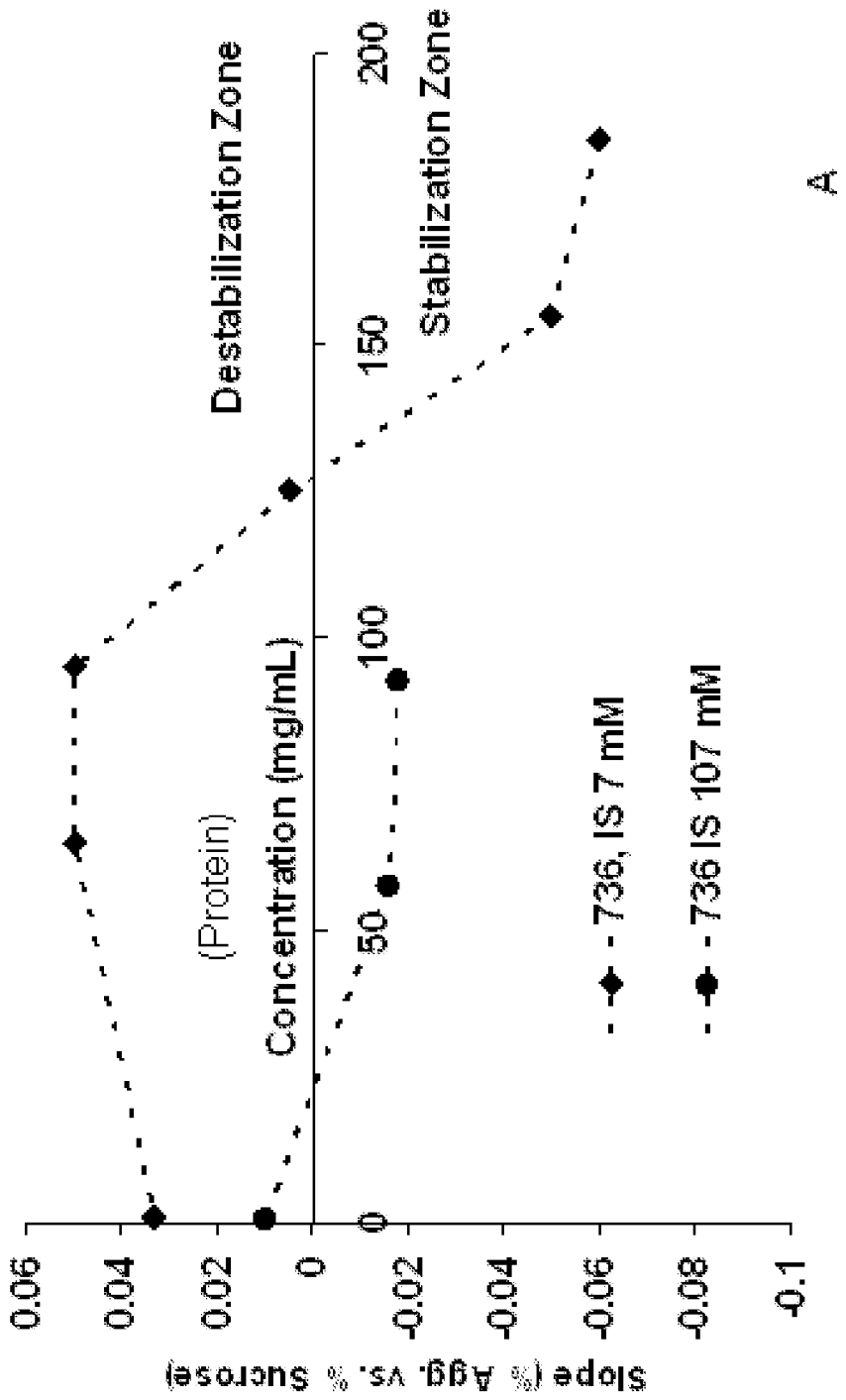
Destabilization Zone
Stabilization Zone
A
(Protein) Concentration (mg/mL)
0
50
100
150
200
- 736, IS 7 mM
- 736 IS 107 mM
Slope (% Agg. vs. % Sucrose)
0.06
0.04
0.02
0
-0.02
-0.04
-0.06
-0.08
-0.1

Figure 3A

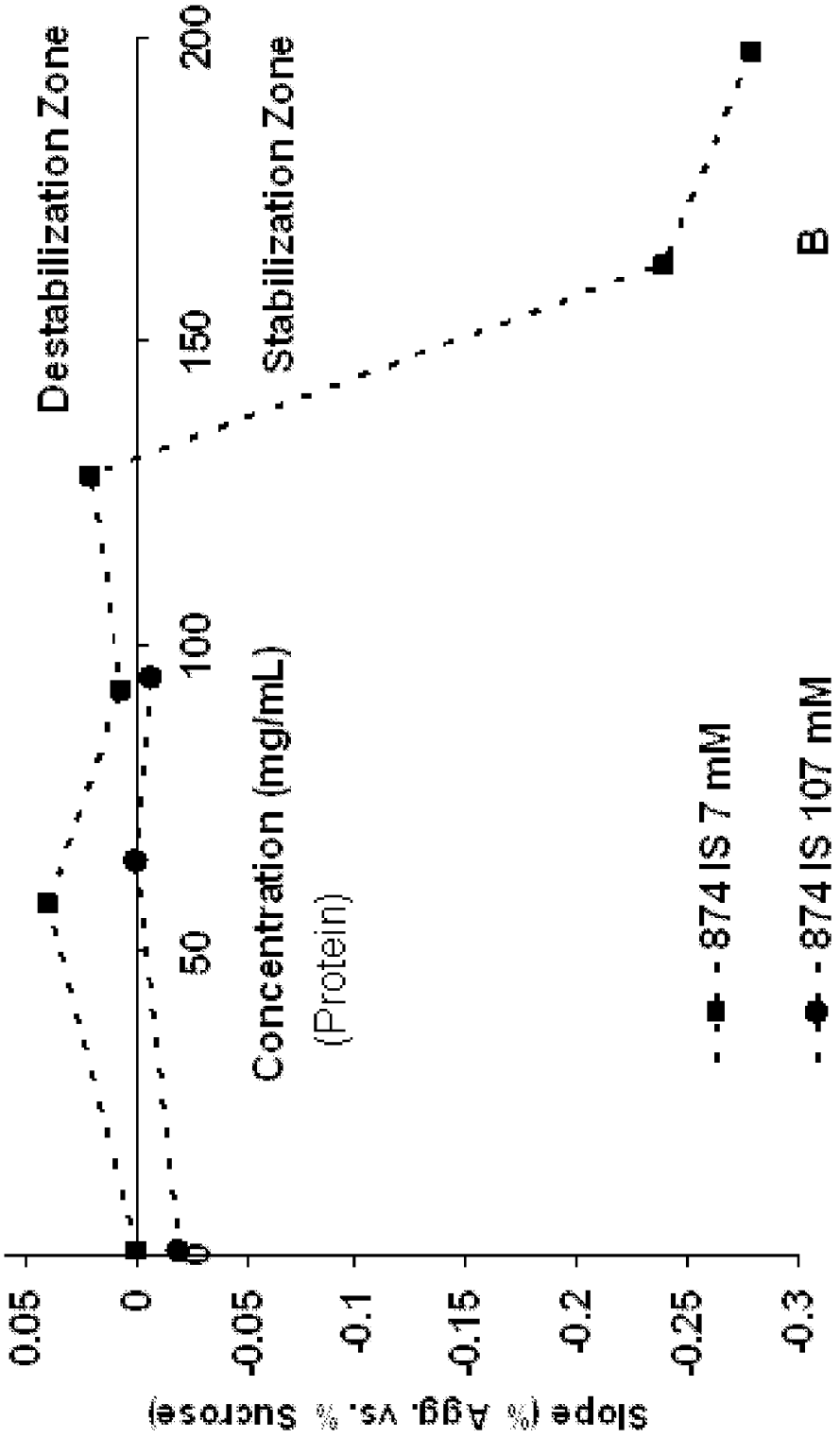
Destabilization Zone
Stabilization Zone
B
Concentration (mg/mL)
(Protein)
874 IS 7 mM
874 IS 107 mM
Slope (% Agg. vs. % Sucrose)
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
0
50
100
150
200

Figure 3B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/71368

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 14/00 (2013.01)
USPC - 530/350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C07K 14/00 (2013.01)
USPC - 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
keyword search, as below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, USPTO PubWest, Google Scholar - Search Terms: increase, improve, enhance, extend, longer, half-life, shelf-life, lifetime, stability, reverse stability, cloud point, flocculate, flocculant, precipitate, aggregate, critical concentration, redissolve, polyol, polyhydric, polyoxalate, polyethylene glycol, peg, polyhydroxy, glycol, protein,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,804,557 A (CLELAND et al.) 8 September 1998 (08.09.1998) Table 1; col 2, In 15-18, col 4, In 65-col 6, In 34; col 3, In 34-46	1-13
Y	US 2009/0298768 A1 (KIM et al.) 3 December 2009 (03.12.2009) Figs. 1, 2; para [0007]; [0008];[0009]; [0018]; [0022];	1-13
Y	US 2010/0278822 A1 (FRAUNHOFER et al.) 4 November 2010 (04.11.2010) abstract; para [0005]; [0127]	10, 11
A	US 2009/0291062 A1 (FRAUNHOFER et al.) 26 November 2009 (26.11.2009)	1-13
A	AHAMED et al., Phase Behavior of an Intact Monoclonal Antibody. Biophys J, 15 July 2007, Vol. 93, No 2, pg. 610-619. Entire document, especially Fig. 7.	1-13

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date orpriority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 April 2013 (09.04.2013)	**26 APR 2013**

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. **571-273-3201**	Lee W. Young **PCT Helpdesk: 571-272-4300** **PCT OSP: 571-272-7774**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/71368

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

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

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

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

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

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

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1 . In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-13, directed to a protein formulation having enhanced shelf-life stability relative to a first protein formulation, wherein the first protein formulation comprises a polyol at a first polyol concentration, the protein formulation comprising:
i) a protein at a concentration below a Reverse Stability Concentration for the protein in the first protein formulation; and
ii) a polyol at a concentration below the first polyol concentration.

- Please see extra sheet for continuation -

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

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

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

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-13

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/71368

Continuation of Box III: Lack of Unity of Invention

Group II: claims 14-24, directed to a method of detecting the Reverse Stability Concentration of a protein in a formulation comprising a polyol, the method comprising:
preparing a first series of formulations comprising a protein at a first protein concentration and a polyol, wherein the polyol concentration is varied over a range;
preparing a second series of formulations comprising a protein at a second protein concentration that is higher than the first protein concentration and a polyol, wherein the polyol concentration is varied over a range, and wherein the concentration of other solutes in the first series of formulations and second series of formulations is the same;
exposing the first series of formulations and the second series of formulations to a test condition;
measuring the level of protein aggregation in the first series of formulations and the second series of formulations after exposure to the test condition; and
determining the change in the level of aggregation in each series of formulations from lowest to highest polyol concentration;
wherein an increase in the level of aggregation in the first series of formulations and a decrease in the level of aggregation in the second series indicates that the Reverse Stability Concentration is between the first protein concentration and the second protein concentration for the protein in the formulation comprising a polyol.

Group III: claims 25-31 , directed to a method for reducing protein aggregation in a formulation comprising a protein and a polyol, the method comprising:
determining a Reverse Stability Concentration for the protein in the formulation, and
reducing the concentration of the polyol in the formulation if the protein concentration
is lower than the Reverse Stability Concentration,
thereby reducing protein aggregation in the formulation.

The inventions listed as Groups I - III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical features of the claims of Groups I-III are indicated in the above Group descriptions.

The only common technical element shared by the above groups is that they are related to a Reverse stability Concentration (RSC) for a protein in a formulation further comprising a polyol. Groups II and III share the common technical element of being related to the determination of said RSC in a formulation comprising a protein and a polyol, or the determination that said RSC exists. Groups I and III share the common technical element of a protein formulation having a protein concentration below the RSC. These common technical elements do not represent an improvement over the combined prior art of US 5,804,557 A to Cleland et al. (hereinafter "Cleland") in view of the article entitled "Phase Behavior of an Intact Monoclonal Antibody" by Ahamed et al. (hereinafter "Ahamed"), as follows:

Regarding the first common technical element, Cleland teaches protein formulations comprising a polyol (aqueous polypeptides.. .admixed with a polyol; abstract).

Regarding the third common technical element, although Ahamed does not specifically recite the presence of a RSC for a protein in a formulation comprising a polyol, Ahamed illustrates a graph showing data for a formulation comprising a protein and a polyol (Fig. 7). Wherein the polyol concentration was gradually increased in a series of dilutions of the protein (pg 615, col 2, para 2). The production of a formulation comprising a polyol and having a protein concentration at a level below the RSC in the formulation is therefore inherent in the range of dilutions produced and illustrated by Ahamed.

Regarding the second common technical element, the claims of Group II are specifically directed to the determination of said RSC in a formulation comprising a protein and a polyol, or the determination that said RSC exists. The independent claims of said Group are claims 14, 15 and 16, which do not improve upon the combined prior art of Cleland and Ahamed, as follows:

- Please see next extra sheet for continuation -

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/71368

Continuation of First Extra Sheet: Lack of Unity of Invention

Regarding claim 14, Cleland teaches a method of determining the stability of a protein in a formulation comprising a polyol (Recombinant human growth homone (hGH) and recombinant human gamma-interferon (hIFN-.gamma.) were formulated with various excipients for analysis of the excipient effects on stabilization in the organic solvent, methylene chloride. Optimum formulations were generally those that yielded the maximum soluble polypeptide concentration and the greatest recovery of native polypeptide after treatment with methylene chloride; col 4, ln 65-col 5, ln 6; the excipient used to stabilize the polypeptide of interest will typically be a polyol; col 3, ln 34-35), the method comprising:

a) preparing a series of formulations comprising a first protein and a polyol, wherein the polyol is varied over a range of concentrations, and the protein is varied over a range of concentrations (col 4, ln 65-col 6, ln 15); and wherein the concentration of other solutes in the formulations is the same (all solutions contained 10 mM NaP04, pH 8; Table I, footnote a);

b) exposing the series of formulations to a test condition (treatment with methylene chloride...both polypeptide formulations were tested for stability in methylene chloride by addition of 100 mu.l of the polypeptide solution to 1 ml of methylene chloride. The mixture was then sonicated for 30 sec. After sonication, the polypeptide was extracted from the organic phase by dilution into 50 ml of excipient-free buffer...the amount of soluble polypeptide recovered was determined by ultraviolet absorbance measurements and the amount of monomelic polypeptide was assessed by size exclusion chromatography; col 5, ln 5-28); and

c) determining a change in the amount of stable protein in each of the series of formulations after exposure to the test condition (Table I); and

d) determining the presence of a change in the level of stability in the series of formulations over the range of polyol concentration (wherein an increase in stability (as recovery of the soluble monomeric form, Table I) can be demonstrated for a range of polyol and protein concentrations (Table I: data for PEG, mw 1000 and no polyol control).

Although Cleland does not expressly recite wherein, in a): the protein concentration in the formulations is constant and the polyol concentration is varied over a range of concentrations, in a related disclosure, Ahamed illustrates a graph showing data for a formulation comprising a protein and a polyol (Fig. 7). Wherein the polyol concentration was gradually increased in a series of dilutions of the protein (pg 615, col 2, para 2). It would have been obvious to a person of ordinary skill in the art to vary both the protein and polyol concentrations systematically and independently in a series of formulations, as suggested by the teaching of Ahamed, in order to determine the most stable protein concentration, based on the teaching of Cleland (The excipient used to stabilize the polypeptide of interest will typically be a polyol...optimal ratios are chosen on the basis of an excipient concentration which allows maximum solubility of polypeptide with minimum denaturation of the polypeptide; col 3, ln 34-46).

Further, although Cleland does not explicitly disclose, in step c): measuring the level of protein aggregation in the series of formulations after exposure to the test condition, Cleland teaches measuring the amount of stable protein, rather than the aggregated protein. It would have been obvious to a person of ordinary skill in the art to specifically determine the amount of protein aggregation, as claimed, as the counterpart of the amount of stable protein determined by Cleland, without undue experimentation, as the two quantities would have obviously summed to the initial amount of protein present in the formulations.

Additionally, although Cleland does not specifically indicate, in step d): wherein an absence of a change in the level of aggregation in the series of formulations over the range of polyol concentrations indicates that the protein concentration is the Reverse Stability Concentration of the protein in the formulation, Cleland teaches wherein an intermediate range of polyol concentration is optimal for protein stability at high concentration for at least one polyol (Table I, data for PEG1000, stability at intermediate PEG1000 concentrations is higher than at either very low or very high concentration). Further, Ahamed illustrates wherein, at a given protein concentration, it is possible to establish that a range of polyol concentrations result in non-precipitated protein, and that this range increases as the concentration of polypeptide decreases (Fig. 7). It would have been obvious to a person of ordinary skill in the art to determine an optimal working concentration for the protein, wherein a range of polyol concentrations resulted in a lack of change of aggregation (stability) of the protein using the method of Cleland and the multiple protein and polyol concentrations taught by Ahamed. It further would have been obvious to determine the polyol concentration range limits based on those acceptable to a patient, such as the maximum concentration which limited irritation or inflammation at an injection site, in order to determine the optimal protein concentration having the same level of aggregation or no aggregation over the range of polyol concentrations in order to provide injection formulations having the greatest stability, and least irritation to the patient, wherein said optimum concentration may have been considered the RSC, based on the fact that said concentration would have been lower than the saturation concentration used by Cleland. Said protein concentration would have been anticipated to be more stable with a lower polyol concentration than a higher polyol concentration based on the teaching of Ahamed, which discloses that, for sufficiently high polyol concentrations, all proteins become unstable and aggregate at any concentration (Fig. 7). Therefore the protein concentration of maximal stability determined according to the combined teachings of Cleland and Ahamed would have fit the claimed description of the Reverse Stability Concentration (instant application, pg. 4, para 1).

Regarding claim 15, Cleland teaches a method of determining the stability of a protein in a formulation comprising a polyol (Recombinant human growth homone (hGH) and recombinant human gamma-interferon (hIFN-.gamma.) were formulated with various excipients for analysis of the excipient effects on stabilization in the organic solvent, methylene chloride. Optimum formulations were generally those that yielded the maximum soluble polypeptide concentration and the greatest recovery of native polypeptide after treatment with methylene chloride; col 4, ln 65-col 5, ln 6; the excipient used to stabilize the polypeptide of interest will typically be a polyol; col 3, ln 34-35), the method comprising:

a and b) preparing at least first and second formulations comprising a first protein and a polyol, wherein the polyol is varied over a range of concentrations, and the protein concentration in at least a second series or formulation is higher than the first protein concentration (the protein is also varied over a range of concentrations; col 4, ln 65-col 6, ln 15); and wherein the concentration of other solutes in the formulations is the same (all solutions contained 10 mM NaP04, pH 8; Table I, footnote a);

c) exposing the series of formulations to a test condition (treatment with methylene chloride...both polypeptide formulations were tested for stability in methylene chloride by addition of 100 mu.l of the polypeptide solution to 1 ml of methylene chloride. The mixture was then sonicated for 30 sec. After sonication, the polypeptide was extracted from the organic phase by dilution into 50 ml of excipient-free buffer...the amount of soluble polypeptide recovered was determined by ultraviolet absorbance measurements and the amount of monomeric polypeptide was assessed by size exclusion chromatography; col 5, ln 5-28); and

d) determining a change in the amount of stable protein in each of the formulations after exposure to the test condition (Table I), wherein an increase in stability (as recovery of the soluble monomeric form, Table I) can be demonstrated for a range of polyol and protein concentrations (Table I: data for PEG, mw 1000 and no polyol control).

- Please see next extra sheet for continuation -

Continuation of Second Extra Sheet: Lack of Unity of Invention

Although Cleland does not expressly recite wherein the first and second protein formulations comprise a series of formulations, in a related disclosure, Ahamed illustrates a graph showing data for a formulation comprising a protein and a polyol (Fig. 7). Wherein the polyol concentration was gradually increased in a series of dilutions of the protein (pg 615, col 2, para 2). It would have been obvious to a person of ordinary skill in the art to vary both the protein and polyol concentrations systematically and independently in a series of formulations, as suggested by the teaching of Ahamed, in order to determine the most stable protein concentration, based on the teaching of Cleland (The excipient used to stabilize the polypeptide of interest will typically be a polyol...optimal ratios are chosen on the basis of an excipient concentration which allows maximum solubility of polypeptide with minimum denaturation of the polypeptide; col 3, ln 34-46). Further, although Cleland does not explicitly disclose, in step c): measuring the level of protein aggregation in the series of formulations after exposure to the test condition, Cleland teaches measuring the amount of stable protein, rather than the aggregated protein. It would have been obvious to a person of ordinary skill in the art to specifically determine the amount of protein aggregation, as claimed, as the counterpart of the amount of stable protein determined by Cleland, without undue experimentation, as the two quantities would have obviously summed to the initial amount of protein present in the formulations.
Additionally, although Cleland does not explicitly recite step e) determining the change in the level of aggregation in each series of formulations from lowest to highest polyol concentration, as in step D, Cleland teaches determining the amount of stable protein in each formulation. It would have been obvious to combine the teaching of Ahamed regarding providing a series of dilutions, and graphing the results versus both polyol and protein concentration would have enabled determining the change in the level of aggregation (stable protein) in each series of formulations from lowest to highest in order to better determine the optimally stable concentration of both a protein and polyol excipient in a formulation, as suggested by Cleland (The excipient used to stabilize the polypeptide of interest will typically be a polyol...optimal ratios are chosen on the basis of an excipient concentration which allows maximum solubility of polypeptide with minimum denaturation of the polypeptide; col 3, ln 34-46).
Furthermore, although neither Cleland nor Ahamed specifically discloses wherein an increase in the level of aggregation in the first series of formulations and a decrease in the level of aggregation in the second series indicates that the Reverse Stability Concentration is between the first protein concentration and the second protein concentration for the protein in the formulation comprising a polyol, the data provided by Cleland indicates an increase in aggregation (decreased stability) in a first formulation, having a lower protein concentration relative to a second formulation having a higher protein concentration (Table I, data for PEG1000). It would have been obvious to a person of ordinary skill in the art that the RSC (optimal protein concentration in the formulation) may have been in between the first protein concentration and the second protein concentration, based on the data of Cleland, wherein the RSC would have been the lowest concentration of protein in the formulation where the protein aggregation decreased. Said concentration would have been obvious to a person of ordinary skill in the art to determine, based on the data of Cleland, in order to provide stable protein formulations comprising polyols for injection, wherein the protein and polyol concentrations were minimized in order to reduce irritation or inflammation at the injection site.

Regarding claim 16, Cleland teaches a method for detecting an optimum concentration of a protein in a formulation comprising a polyol at a given ionic strength (The excipient used to stabilize the polypeptide of interest will typically be a polyol...optimal ratios are chosen on the basis of an excipient concentration which allows maximum solubility of polypeptide with minimum denaturation of the polypeptide; col 3, ln 34-46), the method comprising:
a) preparing a first formulation and a second formulation, wherein the concentration of polyol is lower in the first formulation than in the second formulation (col 4, ln 65-col 6, ln 15); and wherein the concentration of other solutes in the formulations is the same (all solutions contained 10 mM NaP04, pH 8; Table I, footnote a).
b) exposing the formulations to a test condition (treatment with methylene chloride...both polypeptide formulations were tested for stability in methylene chloride by addition of 100 mu.l of the polypeptide solution to 1 ml of methylene chloride. The mixture was then sonicated for 30 sec. After sonication, the polypeptide was extracted from the organic phase by dilution into 50 ml of excipient-free buffer...the amount of soluble polypeptide recovered was determined by ultraviolet absorbance measurements and the amount of monomeric polypeptide was assessed by size exclusion chromatography; col 5, ln 5-28); and
c) measuring the amount of stable protein in the first and second formulations after testing (Table I), wherein greater protein aggregation (lower stable protein recovered) was found in the first formulation, relative to the second (Table I; data for PEG1000 and no polyol control).
Although Cleland does not explicitly recite wherein, in step a), the concentration of the protein in the first formulation and second formulation is the same, in a related disclosure, Ahamed illustrates a graph showing data for a formulation comprising a protein and a polyol (Fig. 7). Wherein the polyol concentration was gradually increased in a series of dilutions of the protein (pg 615, col 2, para 2). It would have been obvious to a person of ordinary skill in the art to vary the polyol concentration for a given protein concentration in a formulation, as taught by Ahamed, in the method of Cleland, in order to be able to compare the effects of changing the polyol on the protein stability at a single protein concentration in order to determine the optimal polyol concentration for that protein concentration in the formulation. Further, although Cleland does not explicitly disclose, in step c): measuring the level of protein aggregation in the series of formulations after exposure to the test condition, Cleland teaches measuring the amount of stable protein, rather than the aggregated protein. It would have been obvious to a person of ordinary skill in the art to specifically determine the amount of protein aggregation, as claimed, as the counterpart of the amount of stable protein determined by Cleland, without undue experimentation, as the two quantities would have obviously summed to the initial amount of protein present in the formulations.
Additionally, although neither Cleland nor Ahamed specifically recites wherein greater protein aggregation in the second formulation as compared to the first formulation indicates that a Reverse Stability Concentration exists for the protein in the formulation comprising the polyol, it would have been obvious to a person of ordinary skill in the art that an optimum protein concentration or optimum range of concentrations in the protein/polyol (PEG1000) formulation existed, based on the teaching of Cleland that shows wherein intermediate protein and polyol (PEG1000) concentrations demonstrated better stability than lower protein and polyol concentrations, or higher protein and polyol concentrations (data for PEG1000; Table I). While Cleland refers to said concentration generally as an optimum concentration (col 3, ln 34-46), it would have been obvious to a person of ordinary skill in the art that said concentration would have been the same as the claimed RSC, and to use formulations comprising said concentration for optimal stability.

Therefore, the inventions of Groups I-III lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.