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WANG W ET AL: "ANTIBODY STRUCTURE, INSTABILITY, AND FORMULATION", JOURNAL OF PHARMACEUTICAL SCIENCES, AMERICAN PHARMACEUTICAL ASSOCIATION, WASHINGTON, US, vol. 96, no. 1, 1 January 2007 (2007-01-01), pages 1-26, XP009084505, ISSN: 0022-3549, DOI: 10.1002/JPS.20727

Fortsættes ...

DESCRIPTION

Field of the Invention

[0001] The present invention relates to pharmaceutical formulations of a pharmaceutically active antigen binding protein, for example a monoclonal antibody. Such formulations comprise, in addition to the antigen binding protein, a buffering agent and a tonicity agent.

Background of the Invention

[0002] The pharmaceutical use of antibodies has increased over the past years. In many instances such antibodies are injected via the intravenous (IV) route. Unfortunately, the amount of antibody that can be injected via the intravenous route is limited by the physico-chemical properties of the antibody, in particularly by its solubility and stability in a suitable liquid formulation and by the volume of the infusion fluid. Alternative administration pathways are subcutaneous or intramuscular injection, which offer potential advantages in terms of patient compliance and ease of administration. These injection pathways require high protein concentration in the final solution to be injected.

[0003] Accordingly, there is a desire to provide highly concentrated, stable pharmaceutical formulations of therapeutically active antigen binding proteins such as antibodies for subcutaneous injection. The advantage of subcutaneous injections is that it allows the medical practitioner to perform it in a rather short intervention with the patient. Moreover the patient can be trained to perform the subcutaneous injection by himself. Such self-administration is particularly useful during maintenance dosing because no hospital care is needed (reduced medical resource utilization). Usually injections via the subcutaneous route are limited to approximately 2 mL. For patients requiring multiple doses, several unit dose formulations can be injected at multiple sites of the body surface.

[0004] Document D2 (April ET AL: "product monograph BENLYSTA TM", 16 April 2014 (2014-04-16), XPO55203701) discloses the product monograph for Benlysta in lyophilized powder for intravenous delivery..

Summary of the Invention

[0005] In one aspect the present disclosure provides a pharmaceutical formulation for an antigen binding protein comprising a buffering agent and a tonicity agent. More particularly, the present disclosure provides about 150 to 250 mg/mL antigen binding protein; about 1 to 100 mM of a buffering agent providing a pH of about 5.0 to about 7.0; and about 70 to 170 mM of a tonicity agent. In one embodiment the antigen binding protein is an anti-BLyS antibody.

[0006] In one aspect the present invention provides a pharmaceutical formulation for a monoclonal antibody comprising 200 mg/mL monoclonal antibody; 10 mM of a Histidine buffering agent; 115 mM of NaCl; 25 mM of Arginine; and 0.01% (w/v) of polysorbate 80; at a pH of 6.0; and wherein the monoclonal antibody comprises heavy and light chains comprising amino acid sequences of SEQ ID NOs: 6 and 7.

[0007] The disclosure provides a pharmaceutical formulation for an antigen binding protein comprising a buffering agent, a stabilizer, a tonicity agent, and a nonionic surfactant. More particularly, the present disclosure provides a pharmaceutical formulation comprising the antigen binding protein, histidine, arginine, NaCl, and polysorbate 80. In one embodiment the antigen binding protein is an anti-BLyS antibody.

[0008] In another aspect the present invention provides a method of treating a disease or condition which is amenable to treatment with an anti-BLyS antibody in a subject comprising administering a formulation according to the present invention in a subject in an amount effective to treat the disease or condition. In one aspect the disease or condition is an autoimmune disease or disorder.

[0009] In another aspect the present invention provides for a kit comprising one or more vials containing the formulation according to the present invention and instructions for subcutaneous administration of the formulation to a patient.

[0010] In another aspect the present invention provides for an injection device comprising a stable anti-BLyS antibody formulation described herein.

[0011] Any references in the description to methods of treatment refer to the compounds, pharmaceutical compositions and medicaments of the present invention for use in a method for treatment of the human (or animal) body by therapy

[0012] In another aspect the present invention provides for a formulation according to the present invention for use in the treatment of disease selected from the group consisting of systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody ("ANCA") vasculitis, lupus nephritis, primary Sjögren's syndrome, chronic immune thrombocytopenia, myasthenia gravis, symptomatic Waldenström's macroglobulinaemia, immune desensitizing of patients awaiting kidney transplant, membranous nephropathy, systemic sclerosis, rheumatoid arthritis, multiple myeloma, multiple sclerosis, and kidney failure.

Brief Description of the Drawings

[0013]

FIG. 1 shows the effect of protein concentration on aggregation rate for Formulation 1.

FIG. 2 shows the turbidity of Formulations 1 and 5 after 5¼ months at 2-8°C.

FIG. 3 shows the relationship of belimumab viscosity to concentration.

FIG. 4 shows the change in % aggregate after 3 months storage at 2-8 °C for various formulations.

FIG. 5 shows the change in % aggregate after 3 months storage at various temperatures and formulations.

FIG. 6 shows the effect of temperature on aggregation rates after 5¼ months at up to 25°C, and shows that the arginine formulation (open squares on the graph) significantly dampens aggregation when compared to Formulation 1 (filled squares).

FIG. 7 shows aggregation rates of Formulation 1 (full squares; 06-C) and Formulation 5 (hollow squares; 06-D) between 125 and 200 mg/mL and -80°C to 40°C after 5¼ Months Storage and how consistently Formulation 5 (dashed lines) shows a lower aggregation rate than Formulation 1 (solid lines).

FIG. 8 shows reducing CGE degradation rates of formulations 1 (06-C) and 5 (06-D) between 125 and 200 mg/mL and -80°C to 40°C after 5¼ months storage.

FIG. 9 shows acidic rates of Formulations 1 (full squares; 06-C) and 5 (hollow squares; 06-D) between 125 and 200 mg/mL and -80°C to 40°C after 5¼ months storage.

FIG. 10 shows belimumab heavy chain oxidation levels in Formulations 1 and 5 between 125 and 200 mg/mL and -80°C to 40°C after 5¼ months storage.

FIG. 11 shows peptide map of belimumab in Formulation 1 at 200 mg/mL after 5¼ months storage.

FIG. 12 shows peptide map of belimumab in Formulation 5 at 200 mg/mL after 5¼ months storage.

FIG. 13 shows peptide map of belimumab samples with different arginine levels.

FIG. 14 shows HTF pH- buffer screening.

FIG. 15 shows two factor interaction - pH x buffer - SEC monomer.

FIG. 16 shows two factor interaction - pH x buffer - clEF main.

FIG. 17 shows viscosity of anti-IL13 antibody at various concentrations.

FIG. 18 shows viscosity (cP) vs. concentration (mg/mL) results for anti-IL13 T=0 samples from shake study.

FIG. 19 shows that 7 day acetate samples were gelled (left). No gel was observed in the succinate or histidine samples (center and right). The 10 day succinate vial was observed to be in a semi-gel state.

FIG. 20 shows a comparison of near-UV circular dichroism spectra for 3 month chemical stability samples.

Detailed Description of the Invention

[0014] It is to be understood that this disclosure is not limited to particular methods, reagents, compounds, compositions, or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a polypeptide" includes a combination of two or more polypeptides, and the like.

[0015] "About" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or ±10%, including ±5%, ±1%, and ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

[0016] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present invention, the preferred materials and methods are described herein. In describing and claiming the present invention, the following terminology will be used.

[0017] In one aspect the present disclosure provides a pharmaceutical formulation for an antigen binding protein comprising a buffering agent, and a tonicity agent. Also disclosed is a pharmaceutical formulation for an antigen binding protein comprising a buffering agent, a stabilizer, a tonicity agent, and a nonionic surfactant. In one embodiment the formulation is lyophilized or spray dried. In certain embodiments the formulation is lyophilized or spray dried and then later reconstituted with a dispersing agent. In one embodiment the dispersing agent is sterile water or "water for injection" (WFI). The antigen binding protein can be further diluted with isotonic saline or other excipients to produce a desirable concentration prior to administration. In one embodiment the formulation is a reconstituted formulation. In another embodiment the formulation is a liquid pharmaceutical formulation.

[0018] The term "pharmaceutical formulation" or "formulation" refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile.

[0019] A "sterile" formulation is aseptic or free from all living microorganisms and their spores.

[0020] In exemplary embodiments of the present invention, the liquid formulations exhibit desirable characteristics, such as desirable viscosity and surface tension characteristics.

[0021] The term "surface tension" refers to the attractive force exerted by the molecules below the surface upon those at the surface/air interface, resulting from the high molecular concentration of a liquid compared to the low molecular concentration of the gas. Liquids with low values of surface tension, such as nonpolar liquids, flow more readily than water. Typically, values of surface tensions are expressed in newtons/meters or dynes/centimeters.

[0022] "Dynamic surface tension" as referred to herein is the surface/air interface and the dynamic interfacial tension to the surface/surface interface. There are a number of alternative methods for measuring dynamic surface tension, for example, captive bubble surface tensionometry or pulsating bubble surface tensionometry.

[0023] The term "viscosity" refers to the internal resistance to flow exhibited by a fluid at a specified temperature; the ratio of shearing stress to rate of shear. A liquid has a viscosity of one poise if a force of 1 dyne/square centimeter causes two parallel liquid surfaces one square centimeter in area and one square centimeter apart to move past one another at a velocity of 1 cm/second. One poise equals one hundred centipoise.

[0024] Disclosed herein, the viscosity of the formulation comprising buffering agent and stabilizer is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 30% compared to the viscosity of the formulation in the absence of buffering agent and stabilizer. Disclosed herein, the viscosity of the formulation comprising buffering agent and stabilizer is less than about 50 cP, less than about 45 cP, less than about 40 cP, less than about 35 cP, less than about 30 cP, less than about 25 cP, less than about 20 cP, less than about 15 cP, or less than about 10 cP.

[0025] When referring to apparent viscosity, it is understood that the value of viscosity is dependent on the conditions under which the measurement was taken, such as temperature, the rate of shear and the shear stress employed. The apparent viscosity is defined as the ratio of the shear stress to the rate of shear applied. There are a number of alternative methods for measuring apparent viscosity. For example, viscosity can be tested by a suitable cone and plate, parallel plate or other type of viscometer or rheometer.

[0026] "Gelation is defined as the process of formation of a stiff network presumably caused by the onset of topological overlaps among polymerizing mAb or filaments as well as the cross-linking and bundling of these filaments. This stiff network manifests as a solution elastic modulus (G') as well as an increase in its inherent viscous modulus (G' ')."

[0027] Disclosed is a method of reducing or inhibiting gelation of a solution comprising utilizing a formulation of the present invention. Disclosed is a method of reducing or inhibiting gelation of a solution comprising a therapeutic protein, the method comprising administering histidine and sodium chloride to the solution.

[0028] "Polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. A polypeptide can be of natural (tissue-derived) origins, recombinant or natural expression from prokaryotic or eukaryotic cellular preparations, or produced chemically via synthetic methods. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid. Non-natural residues are well described in the scientific and patent literature; a few exemplary non-natural compositions useful as mimetics of natural amino acid residues and guidelines are described below. Mimetics of aromatic amino acids can be generated by replacing by, e.g., D- or L-naphylalanine; D- or L-phenylglycine; D- or L-2 thieneylalanine; D- or L-1, -2,3-, or 4-pyreneylalanine; D- or L-3 thieneylalanine; D- or L-(2-pyridinyl)-alanine; D- or L-(3-pyridinyl)-alanine; D- or L-(2-pyrazinyl)-alanine; D- or L-(4-isopropyl)-phenylglycine: D-(trifluoromethyl)-phenylglycine; D-(trifluoromethyl)-phenylalanine: D-p-fluoro-phenylalanine; D- or L-p-biphenylphenylalanine; K- or L-p-methoxy-biphenylphenylalanine: D- or L-2-indole(alkyl)alanines; and, D- or L-alkylainines, where alkyl can be substituted or unsubstituted methyl, ethyl, propyl, hexyl, butyl, pentyl, isopropyl, iso-butyl, sec-isotyl, iso-pentyl, or non-acidic amino acids. Aromatic rings of a non-natural amino acid include, e.g., thiazolyl, thiophenyl, pyrazolyl, benzimidazolyl, naphthyl, furanyl, pyrrolyl, and pyridyl aromatic rings.

[0029] "Peptide" as used herein includes peptides which are conservative variations of those peptides specifically exemplified herein. "Conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include, but are not limited to, the substitution of one hydrophobic residue such as isoleucine, valine, leucine, alanine, cysteine, glycine, phenylalanine, proline, tryptophan, tyrosine, norleucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. Neutral hydrophilic amino acids which can be substituted for one another include asparagine, glutamine, serine and threonine. "Conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide. Such conservative substitutions are within the definition of the classes of the peptides disclosed. The biological activity of the peptides can be determined by standard methods known to those of skill in the art and described herein.

[0030] "Recombinant" when used with reference to a protein indicates that the protein has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein.

[0031] As used herein a "therapeutic protein" refers to any protein and/or polypeptide that can be administered to a mammal to elicit a biological or medical response of a tissue, system, animal or human that is being sought, for instance, by a researcher or clinician. A therapeutic protein may elicit more than one biological or medical response. Furthermore, the term "therapeutically effective amount" means any amount which, as compared to a corresponding subject who has not received such amount, results in, but is not limited to, healing, prevention, or amelioration of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder. The term also includes within its scope amounts effective to enhance normal physiological function as well as amounts effective to cause a physiological function in a patient which enhances or aids in the therapeutic effect of a second pharmaceutical agent.

[0032] All "amino acid" residues identified herein are in the natural L-configuration. In keeping with standard polypeptide nomenclature, abbreviations for amino acid residues are as shown in the following table.

Table 1. Amino acid abbreviations.

1 Letter	3 Letter	Amino Acid
Y	Tyr	L-tyrosine
G	Gly	L-glycine
F	Phe	L-phenylalanine
M	Met	L-methionine
A	Ala	L-alanine
S	Ser	L-serine
I	Ile	L-isoleucine
L	Leu	leucine
T	Thr	L-threonine
V	Val	L-valine
P	Pro	L-proline
K	Lys	L-lysine
H	His	L-histidine
Q	Gln	L-glutamine
E	Glu	L-glutamic acid
W	Trp	L-tryptophan
R	Arg	L-arginine
D	Asp	L-aspartic acid
N	Asn	L-asparagine
C	Cys	L-cysteine.

[0033] It should be noted that all amino acid residue sequences are represented herein by formulae whose left to right orientation is in the conventional direction of amino-terminus to carboxy-terminus.

[0034] Herein disclosed, the polypeptide is an antigen binding protein. Disclosed herein the antigen binding protein is selected from the group consisting of a soluble receptor, antibody, antibody fragment, immunoglobulin single variable domain, Fab, F(ab')2, Fv, disulphide linked Fv, scFv, closed conformation multispecific antibody, disulphide-linked scFv, or diabody.

[0035] The term "antigen binding protein" as used herein refers to antibodies, antibody fragments and other protein constructs which are capable of binding to an antigen.

[0036] The terms Fv, Fc, Fd, Fab, or F(ab)2 are used with their standard meanings (see, e.g., Harlow et al., Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, (1988)).

[0037] A "chimeric antibody" refers to a type of engineered antibody which contains a naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

[0038] A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., Queen et al., Proc. Natl. Acad Sci USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technology, 9:421 (1991)). A suitable human acceptor antibody may be one selected from a conventional database, e.g., the KABAT ™ database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody. The prior art describes several ways of producing such humanized antibodies--see for example EP-A-0239400 and EP-A-054951.

[0039] The term "donor antibody" refers to an antibody (monoclonal, and/or recombinant) which contributes the amino acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to a first immunoglobulin partner, so as to provide the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody.

[0040] The term "acceptor antibody" refers to an antibody (monoclonal and/or recombinant) heterologous to the donor antibody, which contributes all (or any portion, but in some embodiments all) of the amino acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. In certain embodiments a human antibody is the acceptor antibody.

[0041] "CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate). The structure and protein folding of the antibody may mean that other residues are considered part of the antigen binding region and would be understood to be so by a skilled person. See for example Chothia et al., (1989) Conformations of immunoglobulin

hypervariable regions; Nature 342, p 877-883.

[0042] As used herein the term "domain" refers to a folded protein structure which has tertiary structure independent 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. An "antibody single variable domain" is 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 the binding activity and specificity of the full-length domain.

[0043] The phrase "immunoglobulin single variable domain" refers to an antibody variable domain (V_H, V_{HH}, V_L) that specifically binds an antigen or epitope independently of a different V region or domain. An immunoglobulin single variable domain can be present in a format (e.g., homo- or hetero-multimer) with other, different variable regions or variable domains where the other regions or domains are not required for antigen binding by the single immunoglobulin variable domain (i.e., where the immunoglobulin single variable domain binds antigen independently of the additional variable domains). A "domain antibody" or "dAb" is the same as an "immunoglobulin single variable domain" which is capable of binding to an antigen as the term is used herein. An immunoglobulin single variable domain may be a human antibody variable domain, but also includes single antibody variable domains from other species such as rodent (for example, as disclosed in WO 00/29004), nurse shark and Camelid V_{HH} dAbs (nanobodies). Camelid V_{HH} are immunoglobulin single variable domain polypeptides that are derived from species including camel, llama, alpaca, dromedary, and guanaco, which produce heavy chain antibodies naturally devoid of light chains. Such V_{HH} domains may be humanized according to standard techniques available in the art, and such domains are still considered to be "domain antibodies" according to the disclosure. As used herein "V_H includes camelid V_{HH} domains. NARV are another type of immunoglobulin single variable domain which were identified in cartilaginous fish including the nurse shark. These domains are also known as Novel Antigen Receptor variable region (commonly abbreviated to V(NAR) or NARV). For further details see Mol. Immunol. 44, 656-665 (2006) and US20050043519A.

[0044] The term "Epitope-binding domain" refers to a domain that specifically binds an antigen or epitope independently of a different V region or domain, this may be a domain antibody (dAb), for example a human, camelid or shark immunoglobulin single variable domain.

[0045] As used herein, the term "antigen-binding site" refers to a site on a protein which is capable of specifically binding to antigen, this may be a single domain, for example an epitope-binding domain, or it may be paired V_H/V_L domains as can be found on a standard antibody. Disclosed herein, single-chain Fv (ScFv) domains can provide antigen-binding sites.

[0046] The terms "mAbdAb" and dAbmAb" are used herein to refer to antigen-binding proteins disclosed herein. The two terms can be used interchangeably, and are intended to have the same meaning as used herein.

[0047] The pharmaceutical formulation disclosed provides about 150 to 250 mg/mL antigen binding protein; about 1 to 100 mM of a buffering agent providing a pH of about 5.0 to about 7.0; and about 70 to 170 mM of a tonicity agent. Alternatively, the pharmaceutical formulation disclosed provides about 150 to 250 mg/mL antigen binding protein; about 1 to 100 mM of a buffering agent providing a pH of 6.0±0.5; about 1 to 100 mM of a stabilizer; about 90 to 150 mM of a tonicity agent; and about 0.005 to 0.015% (w/v) of a nonionic surfactant. In one embodiment the antigen binding protein is an anti-B Lymphocyte Stimulator (anti-BLyS) protein antibody.

[0048] Also described is a pharmaceutical formulation comprising about 150 to 250 mg/mL antigen binding protein; about 1 to 100 mM histidine at pH of 6.0±0.5; about 70 to 170 mM NaCl. Herein disclosed, the formulation further comprises about 0.005 to 0.03% (w/v) of a nonionic surfactant. Herein disclosed, the formulation further comprises about 0.01 to about 0.1 mM of a metal chelator..

[0049] In one aspect the present invention provides a pharmaceutical formulation for a monoclonal antibody comprising about 200 mg/mL monoclonal antibody; about 10 mM of a Histidine buffering agent; about 115 mM of NaCl; about 25 mM of Arginine; and about 0.01% (w/v) of polysorbate 80, at about a pH of 6.0; and wherein the monoclonal antibody comprises heavy and light chains comprising amino acid sequences of SEQ ID NOs: 6 and 7.

[0050] The pharmaceutical formulation of the present invention may be provided in liquid form or may be provided in lyophilized form.

[0051] The pharmaceutical formulation according to the present invention comprises a buffering agent. Buffering agents include, but are not limited to citric acid, HEPES, histidine, potassium acetate, potassium citrate, potassium phosphate (KH_2PO_4), sodium acetate, sodium bicarbonate, sodium citrate, sodium phosphate (NAH_2PO_4), Tris base, and Tris-HCl. In one embodiment, the buffering agent is histidine. Disclosed, the histidine concentration is about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 mM. Disclosed herein, the histidine concentration is 10±5 mM. Herein disclosed, the histidine concentration is 10±2 mM. In one embodiment, the histidine concentration is about 10 mM Disclosed herein, the histidine concentration is about 15 mM

[0052] As used herein the term "buffering agent providing a pH of about 5.0 to about 7.0" refers to an agent which provides that the solution comprising it resists changes in pH by the action of its acid/base conjugate components. The buffer used in the formulations in accordance with the disclosure may have a pH in the range from about 5.5 to about 6.5, or from about 5.8 to about 6.2. In one embodiment the pH is about 6.0. Herein disclosed, the pH is about 6.250. Examples of buffering agents that will control the pH in this range include acetate, succinate,
gluconate, histidine, citrate, glycylglycine and other organic acid buffers. The most suitable buffer in accordance with the present invention is a histidine buffer, such as e.g. L-histidine.

[0053] A "histidine buffer" is a buffer comprising the amino acid histidine. Examples of histidine buffers include histidine chloride, histidine acetate, histidine phosphate, histidine sulfate. The histidine formulation identified in the examples as being most suitable is a histidine buffer made from 0.65 mg/mL L-histidine, 1.2 mg/mL L-histidine monohydrochloride.

[0054] The pharmaceutical formulation according to the present invention comprises a tonicity agent. Tonicity agents, include, but are not limited to

dextrose, glycerin, mannitol, potassium chloride, and sodium chloride. In one embodiment the tonicity agent is sodium chloride. Disclosed herein, the sodium chloride concentration is about 70 to 170mM; about 90-150mM; or about 115±10 mM. Disclosed herein, the sodium chloride concentration is about 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, or 175 mM. In one embodiment, the sodium chloride concentration is about 115 mM. Herein disclosed, the sodium chloride concentration is 150±10 mM. Disclosed herein, the sodium chloride concentration is about 150 mM.

[0055] By "isotonic" is meant that the formulation has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350 mOsm. Isotonicity can be measured using a vapor pressure or freezing-point depression type osmometer.

[0056] In certain embodiments the pharmaceutical formulation according to the present invention comprises a stabilizer. Stabilizers, include, but are not limited to human serum albumin (hsa), bovine serum albumin (bsa), α-casein, globulins, α-lactalbumin, LDH, lysozyme, myoglobin, ovalbumin, and RNase A. Stabilizers also include amino acids and their metabolites, such as, glycine, alanine (a-alanine, β-alanine), arginine, betaine, leucine, lysine, glutamic acid, aspartic acid, proline, 4-hydroxyproline, sarcosine, γ-aminobutyric acid (GABA), opines (alanopine, octopine, strombine), and trimethylamine N-oxide (TMAO). In one embodiment the stabilizer is an amino acid. In one embodiment the amino acid is arginine. Disclosed herein, the arginine concentration is about 20 to 30 mM. Herein disclosed, the arginine concentration is about 25±2 mM.

[0057] In certain embodiments the pharmaceutical formulation according to the present invention comprises a nonionic surfactant. Nonionic surfactants, include, but are not limited to, polyoxyethylensorbitan fatty acid esters (such as polysorbate 20 and polysorbate 80), polyethylene-polypropylene copolymers, polyethylene-polypropylene glycols, polyoxyethylene-stearates, polyoxyethylene alkyl ethers, e.g. polyoxyethylene monolauryl ether, alkylphenylpolyoxyethylene ethers (Triton-X), polyoxyethylene-polyoxypropylene copolymer (Poloxamer, Pluronic), sodium dodecyl sulphate (SDS). In one embodiment the nonionic surfactant is polysorbate 80. Disclosed herein, the polysorbate 80 concentration is about 0.005 to 0.02% (w/v). In one embodiment, the polysorbate 80 concentration is about 0.01% (w/v). Disclosed herein, the polysorbate 80 concentration is about 0.02% (w/v).

[0058] Herein disclosed, the pharmaceutical formulation comprises a metal chelator. Metal chelators, include, but are not limited to EDTA and EGTA. Disclosed herein the metal chelator is EDTA. Disclosed herein, the EDTA concentration is about 0.01 to about 0.02 mM. Herein disclosed, the EDTA concentration is about 0.05 mM.

[0059] In one embodiment, the antigen binding protein is a monoclonal antibody or fragment thereof. In one embodiment, the monoclonal antibody or fragment thereof is mouse, chimeric, humanized, or fully human. Disclosed herein, the monoclonal antibody or fragment thereof binds to BLyS or IL-13.

[0060] The formulation disclosed comprises the antigen binding protein, histidine, arginine, NaCl, and polysorbate 80. In one aspect the formulation comprises about 200mg/mL antigen binding protein, about 10mM histidine, about 25mM arginine, about 115mM NaCl, and about 0.01% polysorbate 80, at about pH 6.0. In one embodiment, the antigen binding protein binds to BLyS.

[0061] The pharmaceutical formulation disclosed provides about 200 mg/mL antigen binding protein; about 15 mM histidine at a pH of about 6.25; about 150 mM NaCl; about 0.02% (w/v) polysorbate 80; and about 0.05 mM EDTA.

[0062] In one aspect the pharmaceutical formulation of the present invention is stable upon freezing and thawing. A "stable" formulation is one in which all the protein therein essentially retain their physical stability and/or chemical stability and/or biological activity upon storage at the intended storage temperature, e.g. 2-8°C. It is desired that the formulation essentially retains its physical and chemical stability, as well as its biological activity upon storage. The storage period is generally selected based on the intended shelf-life of the formulation. Furthermore, the formulation should be stable following freezing (to, e.g., -70°C.) and thawing of the formulation, for example following 1, 2 or 3 cycles of freezing and thawing. 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. Adv. Drug Delivery Rev. 10: 29-90 (1993), for example. Stability can be measured at a selected temperature for a selected time period. Stability can be evaluated qualitatively and/or quantitatively in a variety of different ways, including evaluation of aggregate formation (for example using size exclusion chromatography, by measuring turbidity, and/or by visual inspection); by assessing charge heterogeneity using cation exchange chromatography or capillary zone electrophoresis; amino-terminal or carboxy-terminal sequence analysis; mass spectrometric analysis; SDS-PAGE analysis to compare reduced and intact antibody; peptide map (for example tryptic or LYS-C) analysis; evaluating biological activity or antigen binding function of the antibody; etc.

[0063] In one embodiment, the pharmaceutical formulation of the present invention is suitable for subcutaneous or intramuscular administration.
"Percent identity" between a query amino acid sequence and a subject amino acid sequence is the "Identities" value, expressed as a percentage, that is calculated by the BLASTP algorithm when a subject amino acid sequence has 100% query coverage with a query amino acid sequence after a pair-wise BLASTP alignment is performed. Such pair-wise BLASTP alignments between a query amino acid sequence and a subject amino acid sequence are performed by using the default settings of the BLASTP algorithm available on the National Center for Biotechnology Institute's website with the filter for low complexity regions turned off. Importantly, a query amino acid sequence may be described by an amino acid sequence identified in one or more claims herein.

[0064] The query sequence may be 100% identical to the subject sequence, or it may include up to a certain integer number of amino acid alterations as compared to the subject sequence such that the % identity is less than 100%. For example, the query sequence is at least 50, 60, 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99% identical to the subject sequence. Such alterations include at least one amino acid deletion, substitution (including conservative and non-conservative substitution), or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the query sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the query sequence or in one or more contiguous groups within the query sequence.

[0065] The % identity may be determined across the entire length of the query sequence, including the CDR(s). Alternatively, the % identity may exclude the CDR(s), for example the CDR(s) is 100% identical to the subject sequence and the % identity variation is in the remaining portion of the query sequence, so that the CDR sequence is fixed/intact.

[0066] In one embodiment, the antigen binding protein is a monoclonal antibody or fragment thereof. In one embodiment, the monoclonal antibody or fragment thereof is mouse, chimeric, humanized, or fully human. In one embodiment, the monoclonal antibody or fragment thereof binds to BLyS (SEQ ID NO: 1) or a hetero- or homo-trimeric form of BLyS, for example, the monoclonal antibody or fragment thereof binds to the soluble form of BLyS (SEQ ID NO: 10). Disclosed herein, the monoclonal antibody comprises heavy and light chain variable regions comprising amino acid sequences that are 90% identical to, or is 91% identical to, or is 92% identical to, or is 93% identical to, or is 94% identical to, or is 95% identical to, or is 96% identical to, or is 97% identical to, or is 98% identical to, or is 99% identical to SEQ ID NO: 2 and 90% identical to, or is 91% identical to, or is 92% identical to, or is 93% identical to, or is 94% identical to, or is 95% identical to, or is 96% identical to, or is 97% identical to, or is 98% identical to, or is 99% identical to SEQ ID NO: 3, respectively, or amino acid sequences that are 90% identical to, or is 91% identical to, or is 92% identical to, or is 93% identical to, or is 94% identical to, or is 95% identical to, or is 96% identical to, or is 97% identical to, or is 98% identical to, or is 99% identical to SEQ ID NO: 4 and 90% identical to, or is 91% identical to, or is 92% identical to, or is 93% identical to, or is 94% identical to, or is 95% identical to, or is 96% identical to, or is 97% identical to, or is 98% identical to, or is 99% identical to SEQ ID NO: 5, respectively. In one embodiment, the monoclonal antibody comprises heavy and light chain variable regions comprising amino acid sequences that are 95% identical to SEQ ID NOs: 2 and 3, respectively, or amino acid sequences that are 95% identical to SEQ ID NOs: 4 and 5, respectively. In one embodiment, the monoclonal antibody comprises heavy and light chain variable regions comprising amino acid sequences that are 90% identical to SEQ ID NOs: 2 and 3, respectively, or amino acid sequences that are 90% identical to SEQ ID NOs: 4 and 5, respectively. In one embodiment, the monoclonal antibody comprises heavy and light chain variable regions comprising amino acid sequences set out in SEQ ID NOs: 2 and 3, respectively, or SEQ ID NOs: 4 and 5, respectively. Disclosed herein, the monoclonal antibody comprises heavy and light chains comprising amino acid sequences that are 90% identical to, or is 91% identical to, or is 92% identical to, or is 93% identical to, or is 94% identical to, or is 95% identical to, or is 96% identical to, or is 97% identical to, or is 98% identical to, or is 99% identical to SEQ ID NO: 6 and 90% identical to, or is 91% identical to, or is 92% identical to, or is 93% identical to, or is 94% identical to, or is 95% identical to, or is 96% identical to, or is 97% identical to, or is 98% identical to, or is 99% identical to SEQ ID NO: 7, respectively, or amino acid sequences that are 90% identical to, or is 91% identical to, or is 92% identical to, or is 93% identical to, or is 94% identical to, or is 95% identical to, or is 96% identical to, or is 97% identical to, or is 98% identical to, or is 99% identical to SEQ ID NO: 8 and 90% identical to, or is 91% identical to, or is 92% identical to, or is 93% identical to, or is 94% identical to, or is 95% identical to, or is 96% identical to, or is 97% identical to, or is 98% identical to, or is 99% identical to SEQ ID NO: 9, respectively. Further disclosed, the monoclonal antibody comprises heavy and light chains comprising amino acid sequences that are 95% identical to SEQ ID NOs: 6 and 7, respectively, or amino acid sequences that are 95% identical to SEQ ID NOs: 8 and 9, respectively. Herein disclosed, the monoclonal antibody comprises heavy and light chains comprising amino acid sequences that are 90% identical to SEQ ID NOs: 6 and 7, respectively, or amino acid sequences that are 90% identical to SEQ ID NOs: 8 and 9, respectively. Disclosed herein, the monoclonal antibody comprises heavy and light chains comprising amino acid sequences set out in

[0067] SEQ ID NOs: 6 and 7, respectively, or SEQ ID NOs: 8 and 9, respectively. Herein disclosed, the monoclonal antibody comprises CDRs comprising amino acid sequences set out in SEQ ID NOs: 11, 12, 13, 14, 15, and 16. Disclosed herein, the anti-BLyS antibody is selected from the group of belimumab, tabalumab, and a mixture thereof. In one embodiment the anti-BLyS antibody comprises the heavy and light chain sequences set out in SEQ ID NOs: 6 and 7, respectively.

[0068] In one embodiment, the pharmaceutical formulation according to the present invention comprises a monoclonal antibody concentration of 200±20 mg/mL. In one embodiment the antibody concentration is about 200 mg/mL. In one embodiment the anti-BLyS antibody is co-administered concomitantly or sequentially with a corticosteroid. In one embodiment, the corticosteroid is selected from the group consisting of prednisone, prednisolone, hydrocortisone, methylprednisolone and dexamethasone. In one embodiment, the corticosteroid is prednisone.

[0069] In one aspect, the present invention provides for a pharmaceutical formulation for the treatment of a disease or disorder amenable to treatment with an anti-BLyS antibody. In one embodiment, the present invention is directed to a method of treating a disease or condition which is amenable to treatment with an anti-BLyS antibody in a subject comprising administering a formulation according to the present invention in a subject in an amount effective to treat the disease or condition. In one embodiment the disease or condition is selected from the group consisting of systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody ("ANCA") vasculitis, lupus nephritis, primary Sjögren's syndrome, chronic immune thrombocytopenia, myasthenia gravis, symptomatic Waldenström's macroglobulinaemia, immune desensitizing of patients awaiting kidney transplant, membranous nephropathy, systemic sclerosis, rheumatoid arthritis, multiple myeloma, multiple sclerosis, and kidney failure. In another embodiment the disease or condition is systemic lupus erythematosus. In another aspect the present invention provides for a formulation for use in the treatment of disease selected from the group consisting of systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody ("ANCA") vasculitis, lupus nephritis, primary Sjögren's syndrome, chronic immune thrombocytopenia, myasthenia gravis, symptomatic Waldenström's macroglobulinaemia, immune desensitizing of patients awaiting kidney transplant, membranous nephropathy, systemic sclerosis, rheumatoid arthritis, multiple myeloma, multiple sclerosis, and kidney failure. In another aspect the present invention provides for a formulation for use in the treatment of systemic lupus erythematosus. Herein disclosed, is the use of a formulation in the preparation of a medicament for the treatment of a disease selected from the group consisting of systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody ("ANCA") vasculitis, lupus nephritis, primary Sjögren's syndrome, chronic immune thrombocytopenia, myasthenia gravis, symptomatic Waldenström's macroglobulinaemia, immune desensitizing of patients awaiting kidney transplant, membranous nephropathy, systemic sclerosis, rheumatoid arthritis, multiple myeloma, multiple sclerosis, and kidney failure. In another aspect the present invention provides for the use of a formulation in the preparation of a medicament for the treatment of systemic lupus erythematosus.

[0070] In one aspect, the present invention provides for a kit comprising one or more vials containing the formulation of the present invention disclosed and instructions for subcutaneous administration of the formulation to a patient. In one embodiment, the kit further comprises an injection device for subcutaneous administration of the formulation to a patient.

[0071] In one embodiment, the present invention is directed to an injection device comprising a stable anti-BLyS antibody formulation described herein. For subcutaneous delivery, the formulation may be administered via a suitable device, such as (but not limited to) a syringe; an injection device (e.g. the INJECT-EASE™ and GENJECT™ device); an infusion pump (such as e.g. Accu-Chek™); an injector pen (such as the GENPEN™; or a needleless device (e.g. MEDDECTOR™ and BIOJECTOR™).

[0072] The pharmaceutical formulation in accordance with the invention is essentially free from visible (human eye inspection) particles. The sub-visible particles (as measured by light obscuration) should fulfill the following criteria: maximum number of particles ≥10 µm per vial->6000; maximum number of particles ≥25 µm per vial->600.

[0073] The pharmaceutical formulation of the pharmaceutically active anti-BLyS antibody in accordance with the invention can be administered as subcutaneous injection, whereby the administration is repeated several times with time intervals of 1, 2, 3, or 4 weeks. In one embodiment the pharmaceutical formulation of the pharmaceutically active anti-BLyS antibody is administered once every week or once every two weeks. The full volume of the injection fluid is in most cases administered within a time period of 1 to 10 minutes, preferably 2 to 6 minutes, most preferably 3±1 minutes.

[0074] For the prevention or treatment of disease, the appropriate dosage of the antibody will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, on the previous therapy, the patient's clinical history and his response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 50 mg/kg of bodyweight or more specifically between about 0.1 mg/kg to 20 mg/kg of bodyweight) of the antibody is a candidate initial dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. More specifically the dosage of the antibody will be in the range from about 0.05 mg antibody/kg of bodyweight to about 10 mg antibody/kg of bodyweight.

[0075] Disclosed herein isan article of manufacture which contains the pharmaceutical formulation disclosed and provides instructions for its use. This article of manufacture comprises a container. Suitable containers include, for example, bottles, vials (e.g. multiple or dual chamber vials), syringes (such as multiple or dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic. The container holds the formulation and the label on, or associated with, the container may indicate directions for use. The container holding the formulation may be a multi-use vial, which allows for repeat administrations (e.g. from 2 to 6 administrations) of the reconstituted formulation. 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.

[0076] The antibody which is formulated in accordance with the present invention is preferably essentially pure and desirably essentially homogeneous. An "essentially pure" antibody means a composition comprising at least about 90% by weight of the antibody, based on total weight of the composition, preferably at least about 95% by weight. An "essentially homogeneous" antibody means a composition comprising at least about 99% by weight of antibody, based on total weight of the composition.

[0077] The invention will be more fully understood by reference to the following Examples. They are merely illustrative and should not be construed as limiting the scope of the invention. Minor variations in procedure, e.g. minor changes in time, temperature, quantity, concentration, scale, etc., are not anticipated to affect the outcome of the experiments. All literature and patent citations are incorporated herein by reference.

[0078] The Examples are further illustrated by the appended FIGS. 1-20.

Examples

Example 1: belimumab formulations

Container Closure

[0079] Schott type I vials with Daikyo D21-7S Flurotec® stoppers and flip off aluminum seals were used in all studies unless otherwise mentioned. This vial and stopper combination is recommended as the Phase 1 configuration. Long term stability samples that were stored >2-8°C used Gerresheimer 1.0 mL long, 29G, thin wall, staked, pre-filled syringes with Stelmi 4800 needle shields and Daikyo W4023 Flurotec® plungers, and were set by vacuum with a nitrogen overlay. Samples < 2-8°C were filled into cryogenic vials. Product Handling Procedures

[0080] Prior to all experiments, belimumab was sterile filtered with a 0.22 µm filter and aseptically filled into the chosen container closures. All stability samples were protected from light during storage.

Excipient Selection

[0081] Multi-compendial excipients, mandatory for GMP BDS and FDP manufacturing, were used where possible in the screening studies, and used for all formulations in the long term stability study.

[0082] Table 2 provides a list of the formulations tested.

Table 2

Identifier	Formulation Description		
	Buffer conc (mM)	Stabilizing excipient conc (mM)	pH
1	10 mM Histidine	140 mM NaCl	6.0
2	10 mM Histidine	280 mM Sucrose	6.0
3	10 mM Histidine	140 mM Sucrose, 70 mM NaCl	6.0
4	10 mM Histidine	5.4 mM $MgCl_2$, 130 mM NaCl	6.0
5	10 mM Histidine	25 mM Arg, 115 mM NaCl	6.0
6	10 mM Histidine	280 mM Sorbitol	6.0

Identifier	Formulation Description		
	Buffer conc (mM)	Stabilizing excipient conc (mM)	pH
7	10 mM Histidine	25 mM Arg, 5.4 mM $MgCl_2$, 105 mM NaCl	6.0
8	10 mM Succinate	140 mM NaCl	6.0

Long Term Stability

Concentration-Dependent Aggregation in Formulation 1

[0083] As expected, aggregation increased with protein concentration (Table 3, Figure 1). The aggregation rate approximately doubles between 100 mg/mL and 260 mg/mL, but even at 260 mg/mL would only lead to approximately 1% increase in aggregation over 3 years at 2-8 °C at 200 mg/mL belimumab. Note the starting amount of aggregation observed by SEC-HPLC increases as protein concentration increases, although only by approximately 0.1% (0 month row of Table 3).

Table 3 Effect of Protein Concentration on % Aggregate

Month	100 mg/mL	140 mg/mL	180 mg/mL	220 mg/mL	260 mg/mL
0	0.47%	0.50%	0.54%	0.58%	0.60%
1	0.47%	0.51%	0.52%	0.58%	0.62%
2	0.54%	0.61%	0.64%	0.75%	0.78%
4	0.53%	0.59%	0.63%	0.72%	0.77%
6	0.58%	0.63%	0.70%	0.76%	0.84%
Rate/Month	0.018%	0.021%	0.027%	0.030%	0.040%

Long Term Formulation Candidate Screen

[0084] Based on the following results, the formulation candidates were narrowed to the Formulations 1 and 5 after evaluation of the 3 month data, and then Formulation 5 was chosen as the final formulation after 5¼ months.

Appearance, pH, and Osmolality

[0085] All samples were opalescent, pale yellow, and free from visible particulate matter at all time points up to 5¼ months. Finished drug product in all eight formulations at all three concentrations most closely matched the Y5 color standard when tested by colorimetry at the initial time point. All FDP samples in the histidine/ NaCl (herein referred to as Formulation 1) and the histidine/ NaCl/ arginine (herein referred to as Formulation 5) formulations also matched the Y5 standard after 3 and 5¼ months of storage at 2-8°C. Turbidity of the samples with sugar stabilizers (sucrose and sorbitol) was significantly lower than all other samples, which ranged from 29-38 NTU at the initial and 3 month time point. Turbidity also increased in the NaCl containing samples as the protein concentration decreased. Only the Formulations 1 and 5 were tested after 5¼ months at 2-8°C, and showed no response to formulation, concentration or time (Figure 2).

Table 4 Turbidity of Long Term Stability Samples after 3 months Storage at 2-8°C

	Turbidity (NTU)					
	High Concentration (~200 mg/mL)		Mid Concentration (~165 mg/mL)		Low Concentration (~125 mg/mL)	
	0 Month	3 Month	0 Month	3 Month	0 Month	3 Month
Hist/NaCl (Formulation 1)	33	33	37	37	37	37
Hist/sucrose (2)	25	23	24	24	22	23
Hist/sucrose/NaCl (3)	30	30	33	33	34	35
Hist/NaCl/$MgCl_2$ (4)	31	31	35	36	36	37
Hist/NaCl/Arg (Formulation 5)	29	28	33	33	33	34
Hist/Sorbitol (6)	27	27	24	26	26	26
Hist/NaCl/Arg/$MgCl_2$ (7)	29	29	32	31	32	33
Succinate/NaCl (8)	31	31	36	35	36	37

[0086] The pH of all samples ranged from 6.1 to 6.3 at the initial time point, and did not shift in Formulations 1 and 5 after 5¼ months (Formulation 5 data shown in Table 13). Osmolality was tested only at the initial time point; all samples were 299 +/- 17 mOsm/kg.

Viscosity and Syringability

[0087] The sugar containing formulations (sucrose, sorbitol) showed the highest viscosities, followed by the succinate/ sodium chloride formulation (Table 5). The rest of the salt containing samples were comparable. Viscosity increased exponentially as protein concentration increased in Formulations 1 and 5 (Figure 3).

Table 5 Viscosity of Long Term Stability Samples at T0

	Viscosity (mm2/s)		
Sample	High Concentration (~200 mg/mL)	Mid Concentration (~165 mg/mL)	Low Concentration (~125 mg/mL)
Hist/NaCl (Formulation 1)	10.2	5.8	3.6
Hist/sucrose	17.4	7.7	4.9
Hist/sucrose/NaCl	13.4	7.9	3.9
Hist/Nacl/$MgCl_2$	12.8	5.6	3.4
Hist/Nacl/Arg (Formulation 5)	13.0	5.8	3.8
Hist/Sorbitol	21.0	7.6	4.3
Hist/NaCl/Arg/$MgCl_2$	12.8	5.6	3.5
Succinate/NaCl	15.3	6.6	3.8

[0088] Syringability, measured as force required to deliver 1 mL through the thin walled 29G needle in 10 seconds, showed similar trends at the initial time point. After 5¼ months, only Formulations 1 and 5 were tested, and there was no significant increase in syringability observed over time at 2-8°C. Syringability over 20 seconds was also tested on one of each syringe at the 5¼ month time point, and delivery force was shown to decrease by up to 40% when delivery time doubled. While not tested, delivery force can also be decreased by increasing needle gauge.

Table 6 Syringability of Long Term Stability Samples at T0 and 5¼ Months

		Syringability (N)		
Sample and Delivery Time	Sample	High Concentration (~200 mg/mL)	Mid Concentration (~165 mg/mL)	Low Concentration (~125 mg/mL)
Initial Time point	Hist/NaCl (Formulation 1)	21.9	14.5	12.0
	Hist/sucrose	31.3	18.2	13.8
	Hist/sucrose/NaCl	25.7	17.5	12.7
	Hist/NaCl/$MgCl_2$	23.8	14.1	11.1
10 Second Delivery	Hist/NaCl/Arg (Formulation 5)	24.4	16.3	11.5
	Hist/Sorbitol	35.7	18.0	12.6
	Hist/NaCl/Arg/$MgCl_2$	25.0	16.1	10.9
	Succinate/N aCl	28.3	14.6	11.7
5¼ month, 2-8 °C Sample	Hist/NaCl (Formulation 1)	24.4	16.1	10.6
10 Second Delivery	Hist/Nacl/Arg (Formulation 5)	24.2	16.7	10.8
5¼ month, 2-8 °C Sample	Hist/NaCl (Formulation 1)	14.4	10.0	9.4
20 Second Delivery	Hist/Nacl/Arg (Formulation 5)	16.2	11.1	6.4

[0089] Forces required to administer drug through seven marketed pen injectors, which are more similar to pre-filled syringes because they require a manual driving force, are similar to the force for belimumab at 200 mg/mL (Table 7). The injection times varied because of different volumes and container diameters, which are listed in Table 7 for comparison. Finally, a University of Nottingham study commissioned by the UK's Department of Trade and Industry has shown that while seated, 59 women between the ages of 16 and 90 were able to apply 53.7 to 237.7 N of downward static force at hip level with their thumb. Although neither the pen injector data nor the force study are perfect correlators for using a pre-filled syringe, both data sets build confidence that the viscosity and syringability of 200 mg/mL belimumab are not prohibitive for manual administration. However, the force required to deliver 200 mg/mL belimumab from a 1 mL long pre-filled syringe through a 29G thin wall needle is at or near the desirable limit for manual injection, and a wider needle would be preferred.

Table 7 Injection Forces of Commercially Available Pens at 80 mm/min

Pen	Provided Needle	Company	Indication	Injection Force (N)	Time to Deliver (seconds)
Gonal-f RFF	Ypsomed Penfine 29Gx1/2"	EMD Serono	Infertility	22.3	5.3
Lantus SoloStar	None (BD recommended)	Sanofi-Aventis	type 2 or Type I diabetes	6.4*	11.3
Lantus SoloStar	BD Microfine 30Gx8mm	Schering Corporation	chronic hepatitis C	21.1	7.1
Lantus SoloStar	None (BD recommended)	Eli Lilly	diabetes	24.9*	7.1
Lantus SoloStar	None (29, 30, 31G recommended)	Amylin Pharmaceuticals Eli Lilly	type 2 diabetes	13.4*	4.1

Pen	Provided Needle	Company	Indication	Injection Force (N)	Time to Deliver (seconds)
Lantus SoloStar	None (Novofine recommended)	Novo Nordisk	GH treatment	13.8*	9.4
Lantus SoloStar	None (BD recommended)	Eli Lilly	osteoporosis	24.1*	5.3
* Delivered using a Ypsomed Penfine 29Gx12.7mm needle					

Size Variants

3 Month SEC-HPLC Data

[0090] Aggregation, seen by SEC-HPLC, was the predominate concentration-dependent pathway for belimumab in all formulations. Percent fragmentation (observed as a back shoulder) was variable between 0.1 and 0.2%, but did not change over time (supported by 5¼ month data).

[0091] After 3 months at 2-8°C, distinct differences in aggregation rate (Figure 4) were observed among belimumab formulated in the eight formulations. Formulation 5 (histidine/NaCl/arginine) showed the lowest rate over three months, particularly at 200 mg/mL (blue in Figure 4). This was supported by the accelerated trends at 200 mg/mL (Figure 5). Succinate was the worst stabilizer at low temperatures, but the best at elevated temperatures. Many of the other salt and sugar formulations, including Formulation 1 (histidine/NaCl), showed similar absolute aggregate percentages and aggregation rates.

5¼ Month SEC-HPLC Data

[0092] Belimumab in Formulations 1 and 5 were evaluated at 5¼ months. The trends observed at 3 months continued, with the arginine containing formulation showing a lower aggregation rate, especially at the highest concentration of 200 mg/mL. Figure 6 shows aggregation rates after 5¼ months at up to 25°C, and shows that the arginine formulation (open squares on the graph) significantly dampens aggregation when compared to Formulation 1 (filled squares). Further analysis of the aggregation rates at various temperatures in Figure 7 shows how consistently Formulation 5 (dashed lines) shows a lower aggregation rate than Formulation 1 (solid lines). If the 2-8 °C aggregation rate observed up to 5¼ months holds through 3 years, FDP would only increase by approximately 1.2%.

CGE

[0093] Reducing capillary gel electrophoresis of Formulations 1 and 5 showed no trends after 5 ¼ months of storage at various temperatures (rates shown in Figure 8). Cross-linking and clipping are therefore not dependent on concentration or formulation.

Charge Heterogeneity

[0094] Ion exchange indicates neither concentration nor the addition of arginine to a histidine buffered salt formulation impacts charge variants (Figure 9). Although acidic variants increase over time at elevated temperatures, little to no change in variants was observed after 5 ¼months.

Oxidation

[0095] No significant changes in oxidation were observed among any of the 8 formulations after 3 months storage at 2-8°C (data not shown). After 5 ¼months, when comparing -80°C and 15°C data, no differences in oxidation were observed between Formulations 1 and 5 or among the three concentrations in either formulation (Figure 10). Approximately 1.0% additional oxidation was observed in all samples after 5¼ months storage at 25°C, and approximately 4.5% additional oxidation was observed at 40°C.

Peptide Mapping

[0096] No differences were observed among the 200 mg/mL Formulation 1 -80°C and 2-8°C samples or the reference standard after 5¼ months (Figure 11). The 25°C sample showed a small increase in T4 deamidation, as expected at accelerated temperatures. The Formulation 5 sample similarly showed T4 deamidation only at 25°C, but also showed inconsistent peak heights in a number of other peptide peaks (T33, T34 and T5 of the heavy chain, T3 of the light chain in Figure 12). These peak heights did not trend with temperature, so digestion interference by arginine was suspected.

[0097] To determine if arginine interference was the cause of the variability, 0, 25 and 50 mM arginine were added to a Formulation 1 sample which had been through the method's desalting step. All three samples were then run through the remaining steps, which include trypsin digestion. The same peptide peaks that showed variability in the stability samples showed responses that correlated with arginine concentration (Figure 13). This indicates the arginine may not always be fully cleared by the desalting step, and explains the temperature independent variation observed in the peptide maps of

samples formulated in Formulation 5. Because no other modifications in the peptide maps were observed, it can be surmised that despite the differences in the maps, the Formulations 1 and 5 had no observable degradation after 5¼ months at 2-8°C, and only minimal degradation after 5¼ months at 25°C.

Potency

[0098] Belimumab remains biologically active after storage at 2-8°C for 3 months in either Formulation 1 or Formulation 5 between 125 and 200 mg/mL, or after 5¼ months in Formulation 5 at 200 mg/mL (Table 8).

Table 8 Relative Potency after Stability at 2-8°C

	% Relative Potency	
	3 Months	5 ¼ Months
200 mg/mL Formulation 5	102%	118%
125 mg/mL Formulation 5	106%	
200 mg/mL Formulation 1	90%	
125 mg/mL Formulation 1	102%	

Evaluation of Freeze/Thaw

[0099] Samples exposed to 5 fast freeze/thaw cycles between -40°C and 2-8°C had a similar amount of aggregation as the -40°C control samples, indicating fast freeze/thaws are not a concern in either Formulation 1 or the Formulation 5 (Table 9).

Table 9 SEC-HPLC Results from belimumab Exposed to Fast Freeze/Thaw

	% Aggregate	
	Formulation 1	Formulation 5
2-8°C ctrl	0.6	0.6
-20°C ctrl	0.8	0.8
-40°C ctrl	0.6	0.7
-80°C control	0.6	0.6
-40/2-8°C 5x Cycle	0.7	0.7

[0100] Samples exposed to 3 slow freeze/thaw cycles showed a 0.2% increase in aggregate level compared to liquid controls (Table 10).

Table 10 SEC-HPLC Results from belimumab Exposed to Slow Freeze/Thaw

Sample	% Aggregate	% Main Peak	% Clip
Formulation 1 Liquid Control	0.7	99.1	0.2
Formulation 1 Slow freeze/thaw	0.9	98.9	0.2
Formulation 5 Liquid Control	0.6	99.2	0.1
Formulation 5 Slow freeze/thaw	0.8	99.1	0.2

DSC

[0101] Calorimetry was used to assess the sub-freezing glass transition (Tg') of each formulation and to determine whether a sub-freezing eutectic was formed. Sodium chloride - water eutectic can form below approximately -21°C, and eutectic crystallization of excipients may affect product quality by introducing crystalline surface interactions and changing the local chemical environment in the freeze concentrate containing protein. Storage below Tg' may improve stability by increasing relaxation time and reducing associated degradation.

[0102] Formulations 1 and 5 of high concentration belimumab had similar behavior with respect to sub-freezing transitions (Table 11). For Formulation 1, Tg' ranged from -23°C (fastest freeze) to - 33°C (slowest freeze). For Formulation 5, Tg' ranged from -22°C (fastest freeze) to -32°C (slowest freeze). For both formulations, a eutectic endotherm was observed only after thermal cycling with multiple annealing steps at -23°C. The eutectic was most likely sodium chloride - water.

[0103] These results indicate that the sub-freezing thermal transitions of these formulations are sensitive to the thermal history of the sample. This is likely due to the high dissolved solids content and the presence of sodium chloride, which can affect Tg' in the protein/amorphous phase. The results, in conjunction with the -80°C and -40°C stability data from Section 5.2, also indicate the BDS storage < -40°C and protected from light is sufficient for belimumab in Formulation 5.

Table 11 DSC Results for belimumab in Formulation 5 & Formulation 1

Sample		Formulation 1:	Formulation 5:
Fast Freeze	Sample mass (mg)	16.5	16.5
	Tg'	-23°C	-22°C

Sample		Formulation 1:	Formulation 5:
	Eutectic endotherm	No eutectic observed	No eutectic observed
Medium freeze to -40°C anneal	Sample mass (mg)	15.7	16.7
	Tg'	-27°C	-26°C
	Eutectic endotherm	No eutectic observed	No eutectic observed
Medium freeze to -23°C anneal	Sample mass (mg)	16.9	16.3
	Tg'	-25°C	-27°C
	Eutectic endotherm	No eutectic observed	No eutectic observed
Slow freeze to -60°C	Sample mass (mg)	17.7	16.3
	Tg'	Weak -33°C	Weak -32°C
	Eutectic endotherm	No eutectic observed	No eutectic observed
Thermal cycling (-80/-23oC with annealing)	Sample mass (mg)	15.3	18.6
	Tg'	After cycling, weak -31°C	After cycling, weak -28°C and - 18°C
	Eutectic endotherm	Between -16°C and -12°C (0.6 J/g)	Between -16°C and -12°C (0.7 J/g)

Evaluation of Shaking

[0104] After 48 hours of shaking at 250 rpm, there was no significant change in purity by SEC-HPLC or turbidity in either the vial or the syringe over the range of polysorbate concentrations studied (Table 12). 0.01% polysorbate 80 was shown to be effective and robust in Formulation 5 as a protectant against shaking in both a vial and a syringe.

Table 12 SEC-HPLC and Turbidity of belimumab in Formulation 5 after Shaking at 250 rpm

		% Main Peak by SEC			Turbidity (NTU)	
Container Closure	Sample	0 hr.	24 hr.	48 hr.	0 hr.	48 hr.
Vial	Control (No Shaking)	99.4	99.3	99.3	37	38
	Low PS80 (0.005%)	99.4	99.3	99.3	35	35
	Target PS80 (0.01%)	99.4	99.3	99.3	33	37
	High PS80 (0.02%)	99.4	99.4	99.4	30	33
Syringe	Control (No Shaking)	99.4	99.3	99.3	37	38
	Low PS80 (0.005%)	99.4	99.3	99.3	35	35
	Target PS80 (0.01%)	99.4	99.3	99.3	33	34
	High PS80 (0.02%)	99.4	99.3	99.4	30	32

Conclusions

[0105] A formulation for subcutaneous administration of belimumab at 200 mg/mL was chosen based on its ability to minimize the primary degradation pathway rates (Formulation 5; 0.65 mg/mL L-histidine, 1.2 mg/mL L-histidine monohydrochloride, 6.7-7.3 mg/mL sodium chloride, 5.3 mg/mL L-arginine hydrochloride, 0.1 mg/mL polysorbate 80, pH 6.0; or, alternatively, 10 mM histidine, 115 mM sodium chloride, 25 mM L-arginine hydrochloride, 0.01% (w/v) polysorbate 80, pH 6.0). The aggregation rate (~0.03%/month at 2-8°C) was shown to increase with belimumab concentration but was inhibited by the use of 25 mM arginine. The deamidation rate was approximately 0.2%/month at 2-8°C. The 200 mg/mL formulation has an acceptable delivery force for manual or autoinjector delivery using a 1 mL long syringe and a 29G thin wall or wider needle. Freeze/thaw profiles and storage at -80°C and -40°C were shown to be acceptable, and the product is not susceptible to shaking stress.

[0106] Long term GMP stability studies were performed on 200 mg/mL belimumab final drug product in Formulation 5 (1.0 mL filled in a 1.0 mL long BD syringe). To date there is 42 months of GMP stability data at intended storage temperature of 2-8°C (Table 14). The results indicate that Formulation 5 provides adequate stability to belimumab with acceptable degradation profiles observed at the intended storage temperature of 2-8°C (Table 14).

Table 13. Long Term Stability of belimumab in Formulation 5 at 200 mg/mL

Time (Month)	Temp (°C)	Appearance	Colorimetry	pH	Turbidity (NTU)	SEC % Aggregate	SEC % Purity	SEC % Fragment	IEC % Acidic	IEC % Main	IEC Basic Shoulder	IEC Basic (w/o shoulder)	RP % HC Oxidation	CGE % Purity	Potency (% Relative Binding)
0	NA	OPF	Y5	6.3	29	0.6	99.2	0.2	12.9	78.5	5.2	8.6	1.8	95.0	
	-80			6.2		0.6	99.4	0	12.6	79.4	5.0	8.0	3.8	93.1	102
	-40			6.2		0.6	99.3	0.1	12.7	79.6	4.8	7.7	3.8	93.8	
	5	OPF	Y5	6.3	29	0.7	99.1	0.2	13.0	79.3	4.9	7.7	2.2	92.4	105
	15		Y5	6.3		1.0	98.8	0.2	15.6	76.9	4.6	7.5	4.1	95.7	
3	25		Y5	6.1		1.2	98.5	0.4	25.7	68.1	3.9	6.2	4.6	93.2	
5.25	-80			6.2		0.6	99.4	0.0	13.9	78.2	4.7	7.9	2.1	94.7	
	-40			6.2		0.7	99.3	0.0	12.2	78.9	5.6	9.0	2.0	95.6	
	5	OPF	Y5	6.2	37	0.7	99.2	0.1	12.9	78.2	5.1	8.8	2.3	94.3	118
	15			6.2		1.0	98.8	0.1	18.1	73.1	5.5	8.8	2.4	92.8	
	25			6.2		1.4	98.2	0.4	34.2	58.5	4.1	7.3	3.3	93.1	
	40			6.2		9.7	88.1	2.2	92.9	2.2	3.3	4.8	6.4	83.7	
9	5	OPF	Y5			0.9	99.1	0.1	14.6	78.3	4.2	7.2	2.6	96.4	

Table 14. GMP Stability Data of 200 mg/mL Belimumab in Formulation 5 at Intended Storage Temperature of2-8°C (1.0 mL in a 1.0 mL Long BD Syringe)

Test (Analytical Method)	Clinical Acceptance Criteria	Time (Months)											
		0	1	2	3	6	9	12	18	24	30	36	42
Appearance [Visual Inspection (Ph. Eur. 2.2.1 and 2.2.2)]	Clear to opalescent, colorless to pale yellow solution, essentially free from foreign particulate matter	OPF	OPF	OPF	OPF	OPF	OPF	OPF	OPF	OPF	OPF	OPF	OPF
Charge Heterogeneity (IE-HPLC)	Main Peak (MP): 64.3 - 89.7%	MP: 75.6	MP: 75.3	MP: 74.9	MP: 74.7	MP: 75.0	MP: 74.1	MP: 72.0	MP: 72.3	MP: 70.7	MP: 69.4	MP: 69.2	MP: 68.3
	Acidic Peaks (AP): Report result (X.X%)	AP: 13.2	AP: 13.4	AP: 13.6	AP: 14.1	AP: 14.1	AP: 14.9	AP: 16.3	AP: 16.2	AP: 17.7	AP: 19.3	AP: 20.1	AP: 20.8
Container Closure Integrity (Dye leak)	Pass							Pass		Pass	Pass	Pass	Pass
Deliverable Volume (USP <1>, Ph. Eur. 2.9.17)	1.0-1.1 mL Maximum volume: X.XX mL	Max: 1.06	Max: 1.06	Max 1.06	Max: 1.06	Max: 1.08	Max 1.07	Max: 1.06	Max: 1.06	Max 1.06	Max: 1.06	Max 1.06	Max 1.07
	Minimum volume: X.XX mL	Min: 1.04	Min: 1.05	Min: 1.05	Min: 1.05	Min: 1.05	Min: 1.05	Min: 1.05	Mini 1.05	Min: 1.05	Min: 1.05	Min: 1.04	Min: 1.05
Injection Forces (Compression Force Test Stand)	Maximum Break Loose Force: Report result (X.X N)	BLF: 7.7	BLF: 7.0	BLF: 7.8	BLF: 7.1	BLF: 7.5	BLF: 7.1	BLF: 8.0	BLF: 8.1	BLF: 7.3	BLF: 6.8	BLF: 8.4	BLF: 9.5
	Maximum Peak Extrusion Force: Report result (X.X N)	PEF: 7.4	PEF: 9.1	PEF: 8.1	PEF: 8.6	PEF: 7.5	PEF: 7.2	PEF: 7.6	PEF: 9.2	PEF: 9.3	PEF: 7.5	PEF: 9.9	PEF: 8.4
pH [pH Electrode (USP <791 >, Ph. Eur. 2.2.3)]	6.0 +/-0.4	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.1	6.0	6.0	6.1	6.1
Potency (Inhibition of Binding)	75 - 133% relative potency	105	105	96	98	98	99	108	93	105	96	98	98
Protein Concentration (A280)	200 +/- 40 mg/mL	200	204	195	202	197	200	198	197	199	197	198	197
Purity (SDS-PAGE: Reduced with Coomassie Blue Stain)	>= 95%	99	98	99	98	99	99	99	99	99	99	99	99
Purity (SEC-	Main Peak (MP):	MP:	MP:	MP:	MP:	MP:	MP:	MP:	MP:	MP:	MP:	MP:	MP:

Test (Analytical Method)	Clinical Acceptance Criteria	Time (Months)											
		0	1	2	3	6	9	12	18	24	30	36	42
HPLC)	>= 95.0%	99.4	99.4	99.2	99.2	99.2	99.1	99.1	99.0	98.9	98.8	98.7	98.8
	Aggregate (AG): Report result (X.X%)	AG: 0.5	AG: 0.6	AG: 0.7	AG: 0.7	AG: 0.8	AG: 0.8	AG: 0.8	AG:0.9	AG:1.0	AG:1.0	AG:1.1	AG:1.0
	Fragment (FG): Report result (X.X%)	FG: 0.1	FG: 0.0	FG: 0.0	FG: 0.0	FG: 0.1	FG: 0.1	FG: 0.1	FG: 0.1	FG: 0.1	FG: 0.1	FG:0.2	FG: 0.1
Sterility (USP <71>, Ph. Eur. 2.6.1)	No growth	No growth											
Subvisible Particulate Matter (USP <788>, Ph. Eur. 2.9.19)	Meets USP <788> and Ph. Eur. 2.9.19	Meets USP <788> and Ph. Eur. 2.9.19						Meets USP <788> and Ph. Eur. 2.9.19		Meets USP <788> and Ph. Eur. 2.9.19:	Meets USP <788> and Ph. Eur. 2.9.19	Meets USP <788> and Ph. Eur. 2.9.19	Meets USP <788> and Ph. Eur. 2.9.19
	<= 6000 particles per container >= 10 um	62						45		290	176	179	293
	<= 600 particles per container >= 25 um	6						0		12	16	7	10
Appearance key: C = Clear, O = Opalescent; L = Colorless, P = Pale yellow, Y = Yellow; F = essentially free from foreign particulate matter; X = other Injection Force: BLF= Break Loose Force, PEF=Peak Extrusion Force,													

Sequence Listing

[0107]

SEQ ID NO: 1 BLyS

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GEGNSSQNSR NKRAVQGPEE TVTQDCLQLI ADSETPTIQK GSYTFVPWLL SFKRGSALEE
KENKILVKET GYFFIYGQVL YTDKTYAMGH LIQRKKVHVF GDELSLVTLF RCIQNMPETL
PNNSCYSAGI AKLEEGDELQ LAIPRENAQI SLDGDVTFFG ALKLL

SEQ ID NO: 2 Belimumab VH

QVQLQQSGAE VKKPGSSVRV SCKASGGTFN NNAINWVRQA PGQGLEWMGG IIPMFGTAKY
SQNFQGRVAI TADESTGTAS MELSSLRSED TAVYYCARSR DLLLFPHHAL SPWGRGTMVT
VSS

SEQ ID NO: 3 Belimumab VL

SSELTQDPAV SVALGQTVRV TCQGDSLRSY YASWYQQKPG QAPVLVIYGK NNRPSGIPDR
FSGSSSGNTA SLTITGAQAE DEADYYCSSR DSSGNHWVFG GGTELTVLG

SEQ ID NO: 4 Tabalumab VH

MKHLWFFLLL VAAPRWVLSQ VQLQQWGAGL LKPSETLSLT CAVYGGSFSG YYWSWIRQPP
GKGLEWIGEI NHSGSTNYNP SLKSRVTISV DTSKNQFSLK LSSVTAADTA VYYCARGYYD
ILTGYYYYFD YWGQGTLVTV SS

SEQ ID NO: 5 Tabalumab VL

EIVLTQSPAT LSLSPGERAT LSCRASQSVS RYLAWYQQKP GQAPRLLIYD ASNRATGIPA
RFSGSGSGTD STLTISSLEP EDFAVYYCQQ RSNWPRTFGQ GTKVEIKRT

SEQ ID NO: 6 Belimumab heavy chain

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SQNFQGRVAI TADESTGTAS MELSSLRSED TAVYYCARSR DLLLFPHHAL SPWGRGTMVT
VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL
QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKK VEPKSCDKTH TCPPCPAPEL
LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS
RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK
SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK

SEQ ID NO: 7 Belimumab light chain

SSELTQDPAV SVALGQTVRV TCQGDSLRSY YASWYQQKPG QAPVLVIYGK NNRPSGIPDR
FSGSSSGNTA SLTITGAQAE DEADYYCSSR DSSGNHWVFG GGTELTVLGQ PKAAPSVTLF
PPSSEELQAN KATLVCLISD FYPGAVTVAW KADSSPVKAG VETTTPSKQS NNKYAASSYL
SLTPEQWKSH RSYSCQVTHE GSTVEKTVAP TECS

SEQ ID NO: 8 Tabalumab heavy chain

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VSSASTKGPS VFPLAPCSRS TSESTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL
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PSVFLFPPKP KDTLMISRTP EVTCVVVDVS QEDPEVQFNW YVDGVEVHNA KTKPREEQFN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKG LPSSIEKTIS KAKGQPREPQ VYTLPPSQEE
MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SRLTVDKSRW
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SEQ ID NO: 9 Tabalumab light chain

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SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSNTLT
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC

SEQ ID NO: 10 Soluble Form of BLyS

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FIYGQVLYTD KTYAMGHLIQ RKKVHVFGDE LSLVTLFRCI QNMPETLPNN SCYSAGIAKL
EEGDELQLAI PRENAQISLD GDVTFFGALK LL

SEQ ID NO: 11 Belimumab CDRH1
GGTFNNNAIN

SEQ ID NO: 12 Belimumab CDRH2
GIIPMFGTAK YSQNFQG

SEQ ID NO: 13 Belimumab CDRH3
SRDLLLFPHH ALSP

SEQ ID NO: 14 Belimumab CDRL1
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GKNNRPS

SEQ ID NO: 16 Belimumab CDRL3
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SEQUENCE LISTING

[0108]

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Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly Lys Leu Leu
        35                  40                  45
Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Cys Cys Leu Thr Val Val
    50                  55                  60
Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp Leu Ala Ser Leu Arg
65                  70                  75                  80
Ala Glu Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Gly Ala Gly
                85                  90                  95
Ala Pro Lys Ala Gly Leu Glu Glu Ala Pro Ala Val Thr Ala Gly Leu
            100                 105                 110
Lys Ile Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Asn
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Ala Leu Glu Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr
            180                 185                 190
Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met
        195                 200                 205
Gly His Leu Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu
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Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu
225                 230                 235                 240
Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly
                245                 250                 255
Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu
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Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45
Gly Gly Ile Ile Pro Met Phe Gly Thr Ala Lys Tyr Ser Gln Asn Phe
    50                  55                  60
Gln Gly Arg Val Ala Ile Thr Ala Asp Glu Ser Thr Gly Thr Ala Ser
65                  70                  75                  80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
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            20                  25                  30
Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr

        35                  40                  45
Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser
    50                  55                  60
Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu
65                  70                  75                  80
Asp Glu Ala Asp Tyr Tyr Cys Ser Ser Arg Asp Ser Ser Gly Asn His
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            20                  25                  30
Pro Ser Glu Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe
        35                  40                  45
Ser Gly Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu
    50                  55                  60
Glu Trp Ile Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro
65                  70                  75                  80
Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln
                85                  90                  95
Phe Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr
            100                 105                 110
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        35                  40                  45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Ser Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Arg
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Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1               5                  10                  15
Ser Val Arg Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Asn Asn Asn
            20                  25                  30
Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45
Gly Gly Ile Ile Pro Met Phe Gly Thr Ala Lys Tyr Ser Gln Asn Phe
    50                  55                  60
Gln Gly Arg Val Ala Ile Thr Ala Asp Glu Ser Thr Gly Thr Ala Ser
65                  70                  75                  80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Ser Arg Asp Leu Leu Leu Phe Pro His His Ala Leu Ser Pro
            100                 105                 110
Trp Gly Arg Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125
Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
    130                 135                 140
Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
        195                 200                 205
Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
    210                 215                 220
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
225                 230                 235                 240
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                245                 250                 255
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            260                 265                 270
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        275                 280                 285
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    290                 295                 300
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
305                 310                 315                 320
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                325                 330                 335
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            340                 345                 350
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
        355                 360                 365
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    370                 375                 380
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr

385                 390                 395                 400
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                405                 410                 415
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            420                 425                 430
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        435                 440                 445
Leu Ser Pro Gly Lys
    450
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<210> 7

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino Acid Sequence identified using molecular biology techniques.

<400> 7

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Ser Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu Gly Gln
 1               5                  10                  15
Thr Val Arg Val Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala
            20                  25                  30
Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35                  40                  45
Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser
    50                  55                  60
Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu
65                  70                  75                  80
Asp Glu Ala Asp Tyr Tyr Cys Ser Ser Arg Asp Ser Ser Gly Asn His
                85                  90                  95
Trp Val Phe Gly Gly Gly Thr Glu Leu Thr Val Leu Gly Gln Pro Lys
            100                 105                 110
Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu Gln
        115                 120                 125
Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro Gly
    130                 135                 140
Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys Ala Gly
145                 150                 155                 160
Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala Ala
                165                 170                 175
Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg Ser
            180                 185                 190
Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr Val
        195                 200                 205
Ala Pro Thr Glu Cys Ser
    210
```

<210> 8

<211> 450

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino Acid Sequence identified using molecular biology techniques.

<400> 8

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Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
 1               5                  10                  15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
            20                  25                  30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
    50                  55                  60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65                  70                  75                  80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95
Arg Gly Tyr Tyr Asp Ile Leu Thr Gly Tyr Tyr Tyr Tyr Tyr Phe Asp Tyr
            100                 105                 110
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115                 120                 125
Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser
    130                 135                 140
Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
145                 150                 155                 160
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
                165                 170                 175
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
            180                 185                 190
Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val
        195                 200                 205
Asp His Lys Pro Ser Gln Thr Lys Val Asp Lys Arg Val Glu Ser Lys
    210                 215                 220
Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly
225                 230                 235                 240
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu
            260                 265                 270
Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg
    290                 295                 300
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320
Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu
                325                 330                 335
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350
Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp
                405                 410                 415
Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu
        435                 440                 445
Gly Lys
    450
```

<210> 9

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino Acid Sequence identified using molecular biology techniques.

<400> 9

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Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1               5                  10                  15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Arg Tyr
            20                  25                  30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Ser Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Arg
                85                  90                  95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175
Asn Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205
Phe Asn Arg Gly Glu Cys
    210
```

<210> 10

<211> 152

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino Acid Sequence identified using molecular biology techniques.

<400> 10

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Ala Val Gln Gly Pro Glu Glu Thr Val Thr Gln Asp Cys Leu Gln Leu
 1               5                  10                  15
Ile Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys Gly Ser Tyr Thr Phe
            20                  25                  30
Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser Ala Leu Glu Glu Lys
        35                  40                  45
Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr Phe Phe Ile Tyr Gly
    50                  55                  60
Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met Gly His Leu Ile Gln
65                  70                  75                  80
Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu
                85                  90                  95
Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu Pro Asn Asn Ser Cys
            100                 105                 110
Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu Leu Gln Leu
        115                 120                 125
Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu Asp Gly Asp Val Thr
    130                 135                 140

Phe Phe Gly Ala Leu Lys Leu Leu
145                 150
```

<210> 11

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino Acid Sequence identified using molecular biology techniques.

<400> 11

```
Gly Gly Thr Phe Asn Asn Asn Ala Ile Asn
 1               5                  10
```

<210> 12

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino Acid Sequence identified using molecular biology techniques.

<400> 12

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Gly Ile Ile Pro Met Phe Gly Thr Ala Lys Tyr Ser Gln Asn Phe Gln
 1               5                  10                  15
Gly
```

<210> 13

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino Acid Sequence identified using molecular biology techniques.

<400> 13

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Ser Arg Asp Leu Leu Leu Phe Pro His His Ala Leu Ser Pro
```

```
 1               5                   10
```

<210> 14
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino Acid Sequence identified using molecular biology techniques.

<400> 14
```
Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala Ser
 1               5                   10
```

<210> 15
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino Acid Sequence identified using molecular biology techniques.

<400> 15
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Gly Lys Asn Asn Arg Pro Ser
 1               5
```

<210> 16
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino Acid Sequence identified using molecular biology techniques.

<400> 16
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Ser Ser Arg Asp Ser Ser Gly Asn His Trp Val
 1               5                   10
```

REFERENCES CITED IN THE DESCRIPTION

Cited references

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Patent documents cited in the description

- EP0239400A [0038]
- EP0554951A [0035]
- WO0029004A [0043]
- US20050043519A [0043]
- WO61994427A [0108]
- WO62093734A [0108]
- WO62095181A [0108]

Non-patent literature cited in the description

- **APRIL et al.**product monograph BENLYSTA TM, 2014, [0004]
- **HARLOW et al.**Antibodies A Laboratory ManualCold Spring Harbor Laboratory19880000 [0035]
- **QUEEN et al.**Proc. Natl. Acad Sci USA, 1989, vol. 86, 10029-10032 [0038]
- **HODGSON et al.**Bio/Technology, 1991, vol. 9, 421- [0038]
- **KABAT et al.**Sequences of Proteins of Immunological InterestU.S. Department of Health and Human Services, National Institutes of Health19870000 [0041]
- **CHOTHIA et al.**Conformations of immunoglobulin hypervariable regionsNature, 1989, vol. 342, 877-883 [0041]
- Mol. Immunol., 2006, vol. 44, 656-665 [0043]

- Peptide and Protein Drug DeliveryMarcel Dekker, Inc.19910000247-301 [0062]
- **JONES, A.**Adv. Drug Delivery Rev., 1993, vol. 10, 29-90 [0062]

Patentkrav

1. Farmaceutisk formulering til et monoklonalt antistof omfattende:
a. 200 mg/mL monoklonalt antistof;
b. 10 mM af et histidin-buffermiddel;
c. 115 mM NaCl;
d. 25 mM arginin; og
e. 0,01% (v/v) polysorbat 80;
ved pH-værdi 6,0; og
hvor det monoklonale antistof omfatter tunge og lette kæder omfattende aminosyresekvenser med SEQ ID NOs: 6 og 7.

2. Farmaceutisk formulering ifølge krav 1, hvor formuleringen omfatter:
a. 200 mg/mL monoklonalt antistof;
b. 0,65 mg/ml L-histidin;
c. 1,2 mg/ml L-histidinmonohydrochlorid
d. 6,7 - 7,3 mg/ml NaCl;
d. 5,3 mg/ml L-argininhydrochlorid; og
e. 0,1 mg/ml polysorbat 80;
ved pH-værdi 6,0.

3. Farmaceutisk formulering ifølge et hvilket som helst foregående krav, som er stabil efter nedfrysning og optøning.

4. Farmaceutisk formulering ifølge et hvilket som helst foregående krav til subkutan eller intramuskulær indgivelse.

5. Farmaceutisk formulering ifølge et hvilket som helst foregående krav til subkutan indgivelse.

6. Farmaceutisk formulering ifølge et hvilket som helst foregående krav i flydende, rekonstitueret, lyofiliseret eller sprøjtetørret form.

7. Farmaceutisk formulering ifølge et hvilket som helst foregående krav i flydende form.

8. Injektionsindretning omfattende den farmaceutiske formulering ifølge et hvilket som helst foregående krav.

9. Kit omfattende et eller flere hætteglas omfattende den farmaceutiske formulering ifølge et af kravene 1-7 og instruktioner til subkutan indgivelse af den farmaceutiske formulering til en patient.

10. Kit ifølge krav 9 og yderligere omfattende en injektionsindretning omfattende den farmaceutiske formulering.

11. Farmaceutisk formulering, injektionsindretning eller kit ifølge et hvilket som helst foregående krav til anvendelse ved behandling af en sygdom eller lidelse, der er tilgængelig for behandling med et anti-BLyS-antistof.

12. Farmaceutisk formulering til anvendelse ifølge krav 11, hvor formuleringen indgives en gang hver uge.

13. Farmaceutisk formulering, injektionsindretning eller kit til anvendelse ifølge et af kravene 11-12, hvor den farmaceutiske formulering co-indgives samtidigt eller sekventielt med et corticosteroid.

14. Farmaceutisk formulering, injektionsindretning eller kit til anvendelse ifølge et af kravene 11-13 til anvendelse ved behandling af en sygdom udvalgt fra gruppen bestående af systemisk lupus erythematosus, anti-neutrofil cytoplasmisk antistof ("ANCA") vaskulitis, lupus nefritis, primært Sjögrens syndrom, kronisk immun trombocytopeni, myasthenia gravis, symptomatisk Waldenströms makroglobulinæmi, immun desensibilisering af patienter, der venter på nyretransplantation, membranøs nefopati, systemisk sklerose, rheumatoid arthritis, multipelt myelom, multipel sklerose og nyresvigt.

15. Farmaceutisk formulering, injektionsindretning eller kit til anvendelse ifølge et af kravene 11-14 til anvendelse ved behandling af systemisk lupus erythematosus.

16. Farmaceutisk formulering, injektionsindretning eller kit til anvendelse ifølge et af kravene 11-14 til anvendelse ved behandling af Sjögrens syndrom.

DRAWINGS

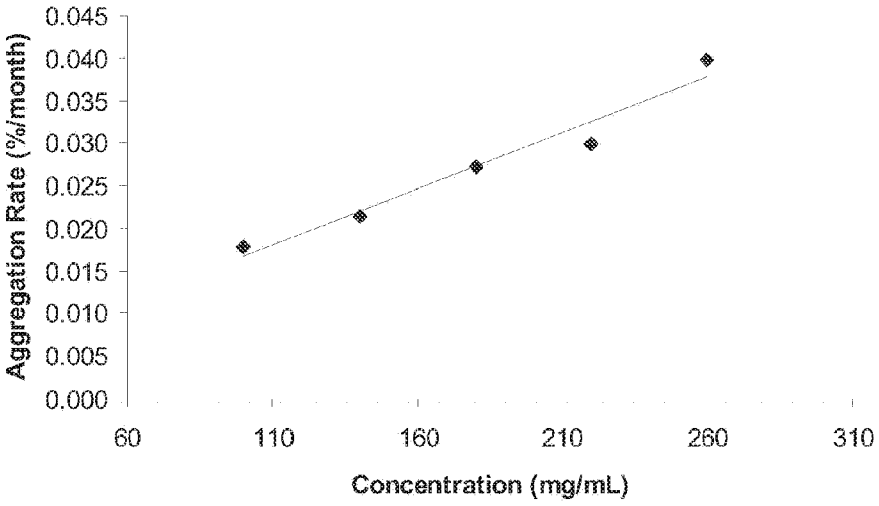


Figure 1

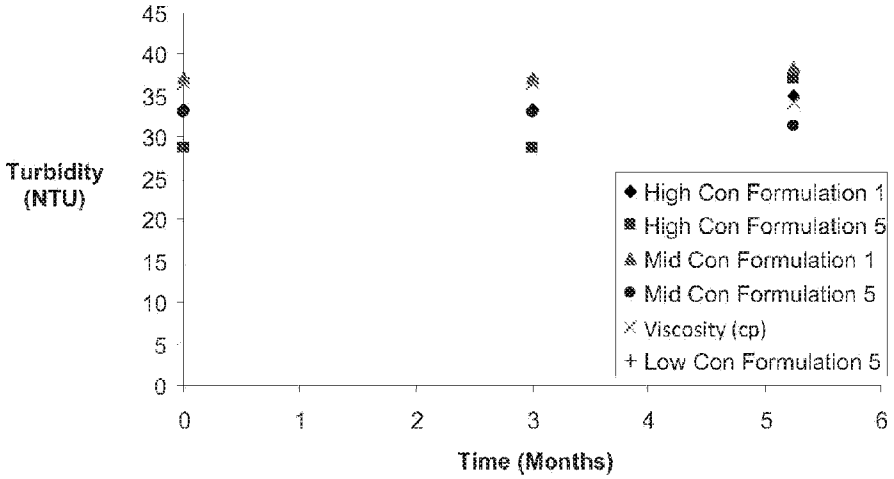

Turbidity (NTU)
45
40
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
Time (Months)
High Con Formulation 1
High Con Formulation 5
Mid Con Formulation 1
Mid Con Formulation 5
Viscosity (cp)
Low Con Formulation 5

Figure 2

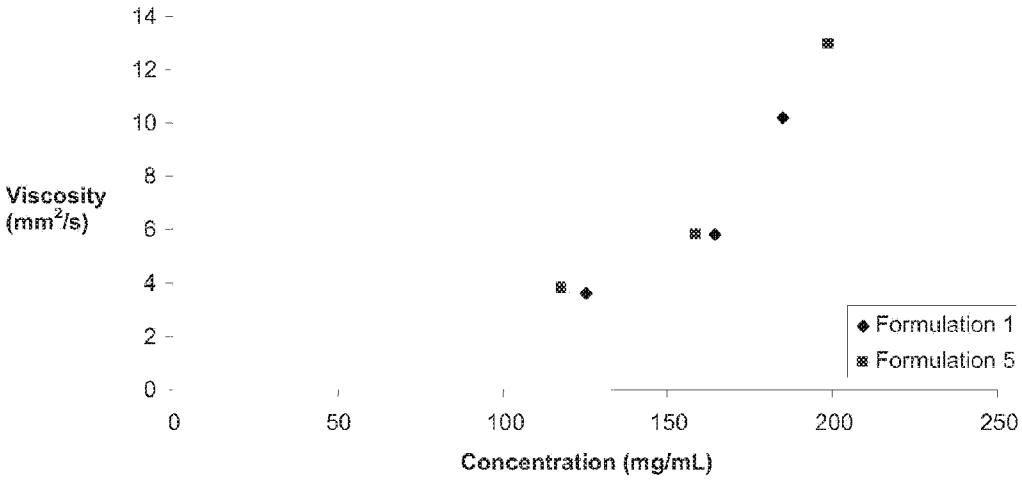

14
12
10
8
6
4
2
0
Viscosity (mm²/s)
0
50
100
150
200
250
Concentration (mg/mL)
Formulation 1
Formulation 5

Figure 3

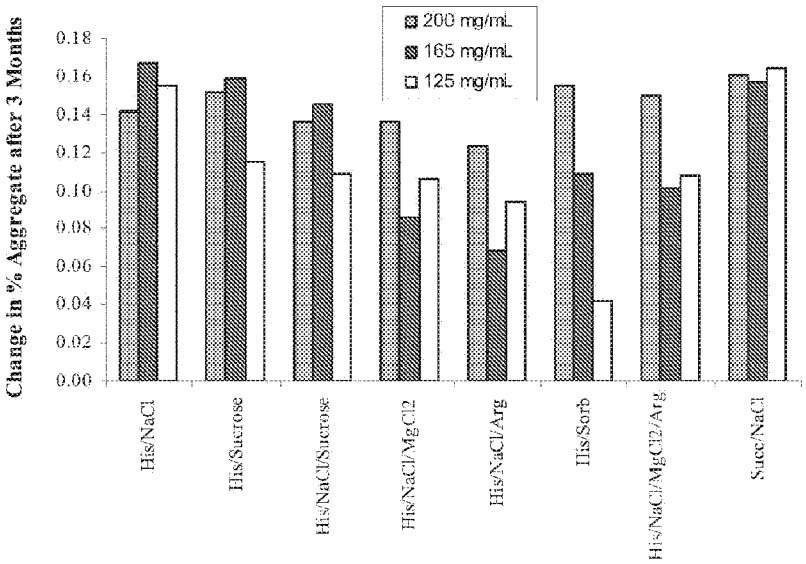

Change in % Aggregate after 3 Months
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
200 mg/mL
165 mg/mL
125 mg/mL
His/NaCl
His/Sucrose
His/NaCl/Sucrose
His/NaCl/MgCl2
His/NaCl/Arg
His/Sorb
His/NaCl/MgCl2/Arg
Succ/NaCl

Figure 4

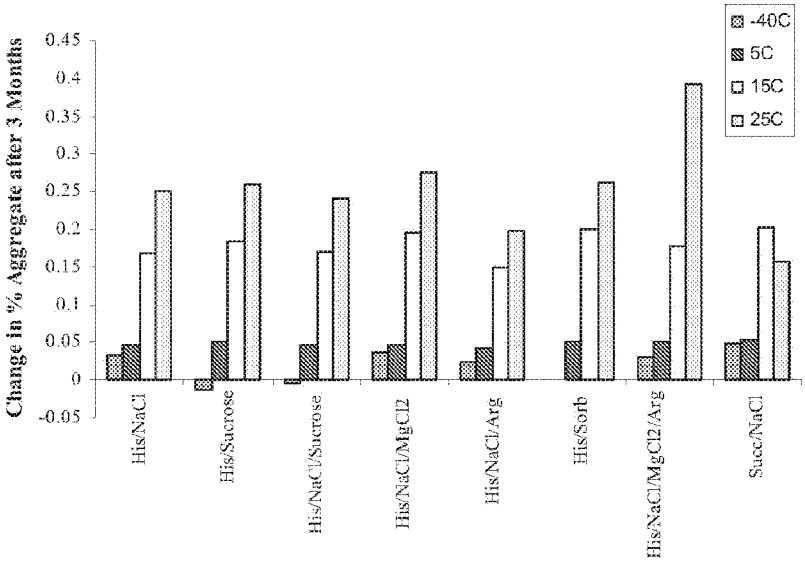

Change in % Aggregate after 3 Months
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-40C
5C
15C
25C
His/NaCl
His/Sucrose
His/NaCl/Sucrose
His/NaCl/MgCl2
His/NaCl/Arg
His/Sorb
His/NaCl/MgCl2/Arg
Succ/NaCl

Figure 5

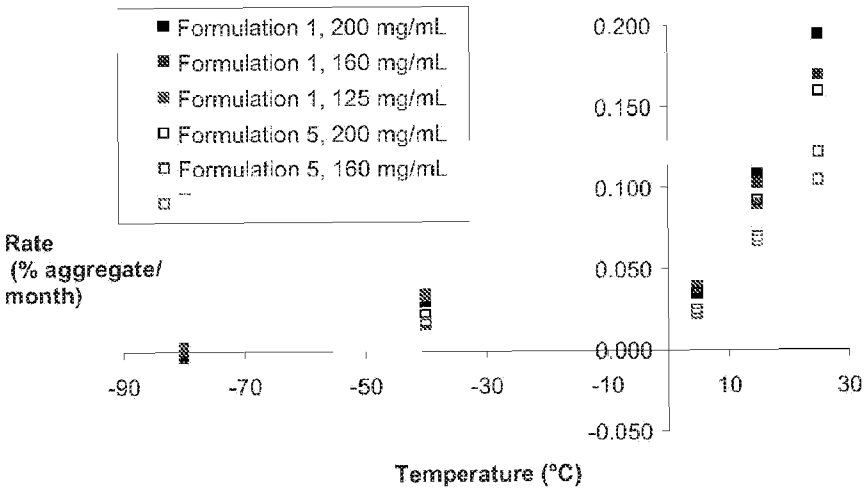
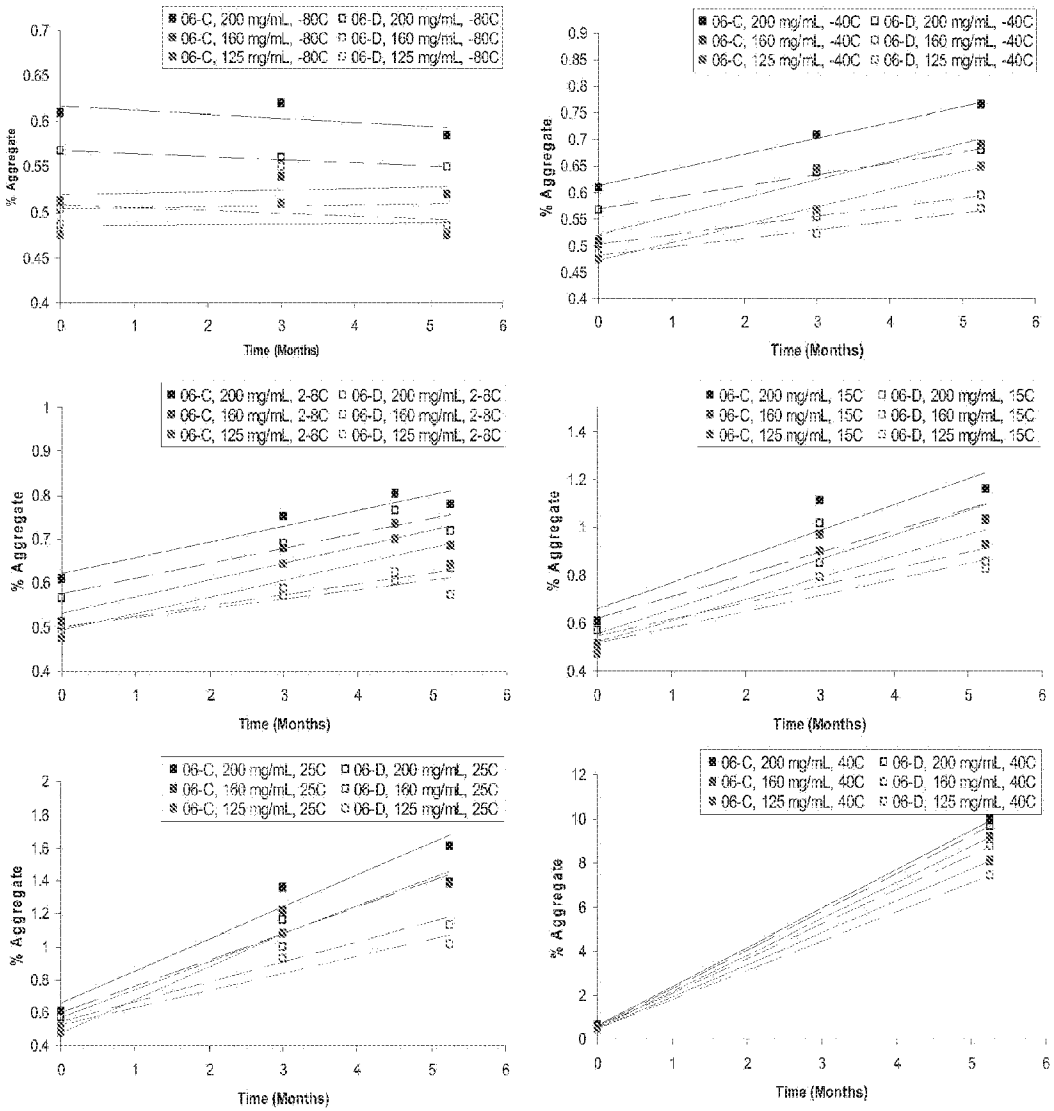

Formulation 1, 200 mg/mL
Formulation 1, 160 mg/mL
Formulation 1, 125 mg/mL
Formulation 5, 200 mg/mL
Formulation 5, 160 mg/mL
Rate (% aggregate/ month)
0.200
0.150
0.100
0.050
0.000
-0.050
-90
-70
-50
-30
-10
10
30
Temperature (°C)

Figure 6


06-C, 200 mg/mL, -80C
06-D, 200 mg/mL, -80C
06-C, 160 mg/mL, -80C
06-D, 160 mg/mL, -80C
06-C, 125 mg/mL, -80C
06-D, 125 mg/mL, -80C
% Aggregate
Time (Months)
06-C, 200 mg/mL, -40C
06-D, 200 mg/mL, -40C
06-C, 160 mg/mL, -40C
06-D, 160 mg/mL, -40C
06-C, 125 mg/mL, -40C
06-D, 125 mg/mL, -40C
% Aggregate
Time (Months)
06-C, 200 mg/mL, 2-8C
06-D, 200 mg/mL, 2-8C
06-C, 160 mg/mL, 2-8C
06-D, 160 mg/mL, 2-8C
06-C, 125 mg/mL, 2-8C
06-D, 125 mg/mL, 2-8C
% Aggregate
Time (Months)
06-C, 200 mg/mL, 15C
06-D, 200 mg/mL, 15C
06-C, 160 mg/mL, 15C
06-D, 160 mg/mL, 15C
06-C, 125 mg/mL, 15C
06-D, 125 mg/mL, 15C
% Aggregate
Time (Months)
06-C, 200 mg/mL, 25C
06-D, 200 mg/mL, 25C
06-C, 160 mg/mL, 25C
06-D, 160 mg/mL, 25C
06-C, 125 mg/mL, 25C
06-D, 125 mg/mL, 25C
% Aggregate
Time (Months)
06-C, 200 mg/mL, 40C
06-D, 200 mg/mL, 40C
06-C, 160 mg/mL, 40C
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06-D, 125 mg/mL, 40C
% Aggregate
Time (Months)

Figure 7

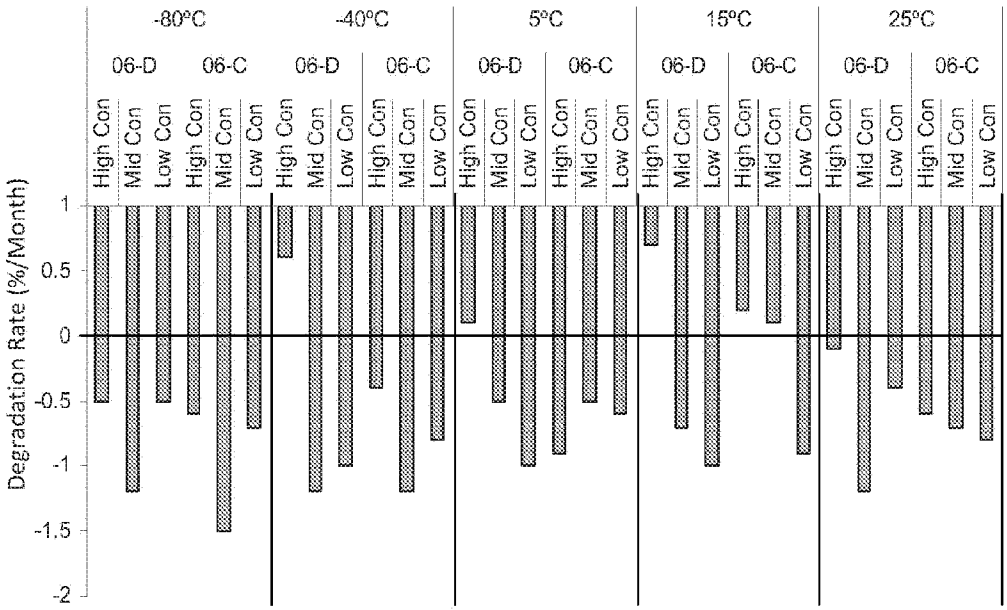

-80°C
-40°C
5°C
15°C
25°C
06-D
06-C
High Con
Mid Con
Low Con
Degradation Rate (%/Month)
1
0.5
0
-0.5
-1
-1.5
-2

Figure 8

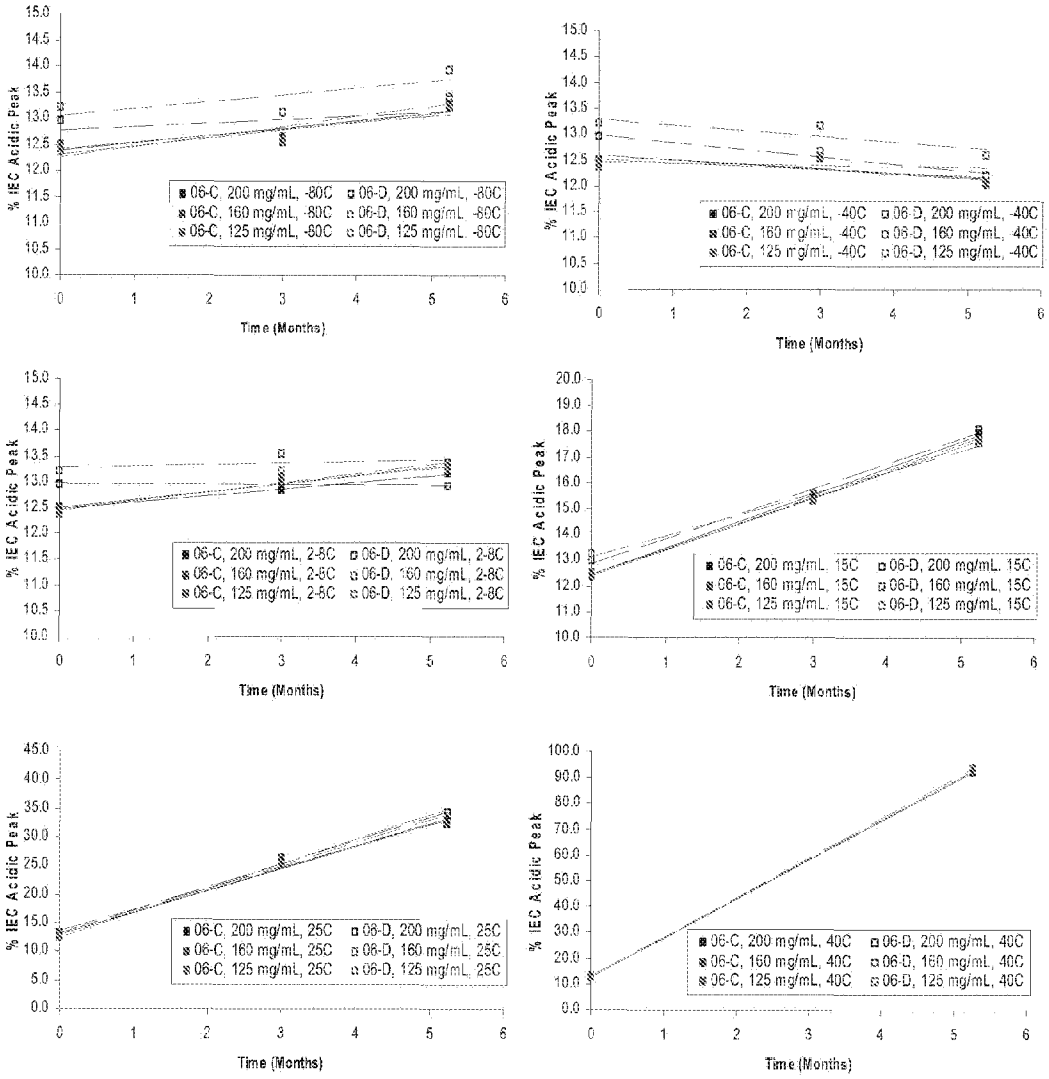

% IEC Acidic Peak
Time (Months)
06-C, 200 mg/mL, -80C
06-D, 200 mg/mL, -80C
06-C, 160 mg/mL, -80C
06-D, 160 mg/mL, -80C
06-C, 125 mg/mL, -80C
06-D, 125 mg/mL, -80C
06-C, 200 mg/mL, -40C
06-D, 200 mg/mL, -40C
06-C, 160 mg/mL, -40C
06-D, 160 mg/mL, -40C
06-C, 125 mg/mL, -40C
06-D, 125 mg/mL, -40C
06-C, 200 mg/mL, 2-8C
06-D, 200 mg/mL, 2-8C
06-C, 160 mg/mL, 2-8C
06-D, 160 mg/mL, 2-8C
06-C, 125 mg/mL, 2-8C
06-D, 125 mg/mL, 2-8C
06-C, 200 mg/mL, 15C
06-D, 200 mg/mL, 15C
06-C, 160 mg/mL, 15C
06-D, 160 mg/mL, 15C
06-C, 125 mg/mL, 15C
06-D, 125 mg/mL, 15C
06-C, 200 mg/mL, 25C
06-D, 200 mg/mL, 25C
06-C, 160 mg/mL, 25C
06-D, 160 mg/mL, 25C
06-C, 125 mg/mL, 25C
06-D, 125 mg/mL, 25C
06-C, 200 mg/mL, 40C
06-D, 200 mg/mL, 40C
06-C, 160 mg/mL, 40C
06-D, 160 mg/mL, 40C
06-C, 125 mg/mL, 40C
06-D, 125 mg/mL, 40C

Figure 9

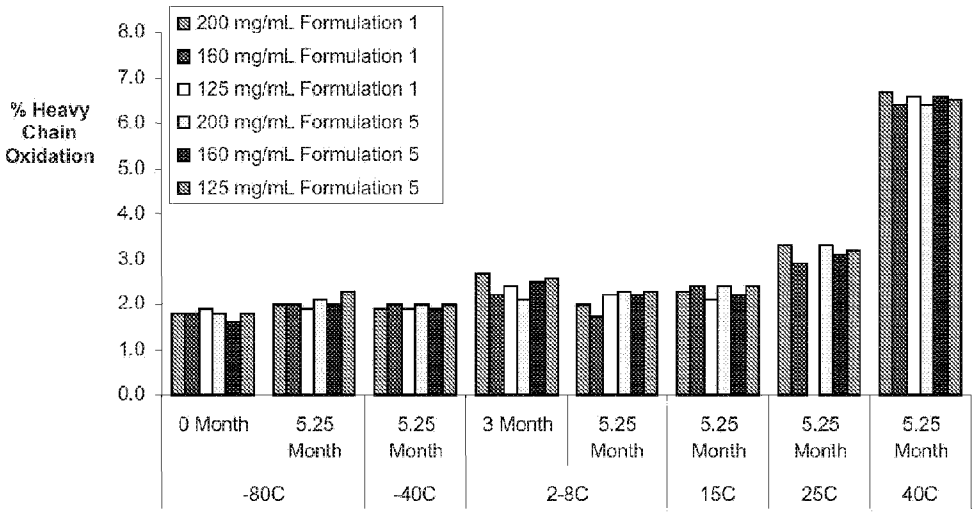

% Heavy Chain Oxidation
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
200 mg/mL Formulation 1
160 mg/mL Formulation 1
125 mg/mL Formulation 1
200 mg/mL Formulation 5
160 mg/mL Formulation 5
125 mg/mL Formulation 5
0 Month
5.25 Month
5.25 Month
3 Month
5.25 Month
5.25 Month
5.25 Month
5.25 Month
-80C
-40C
2-8C
15C
25C
40C

Figure 10

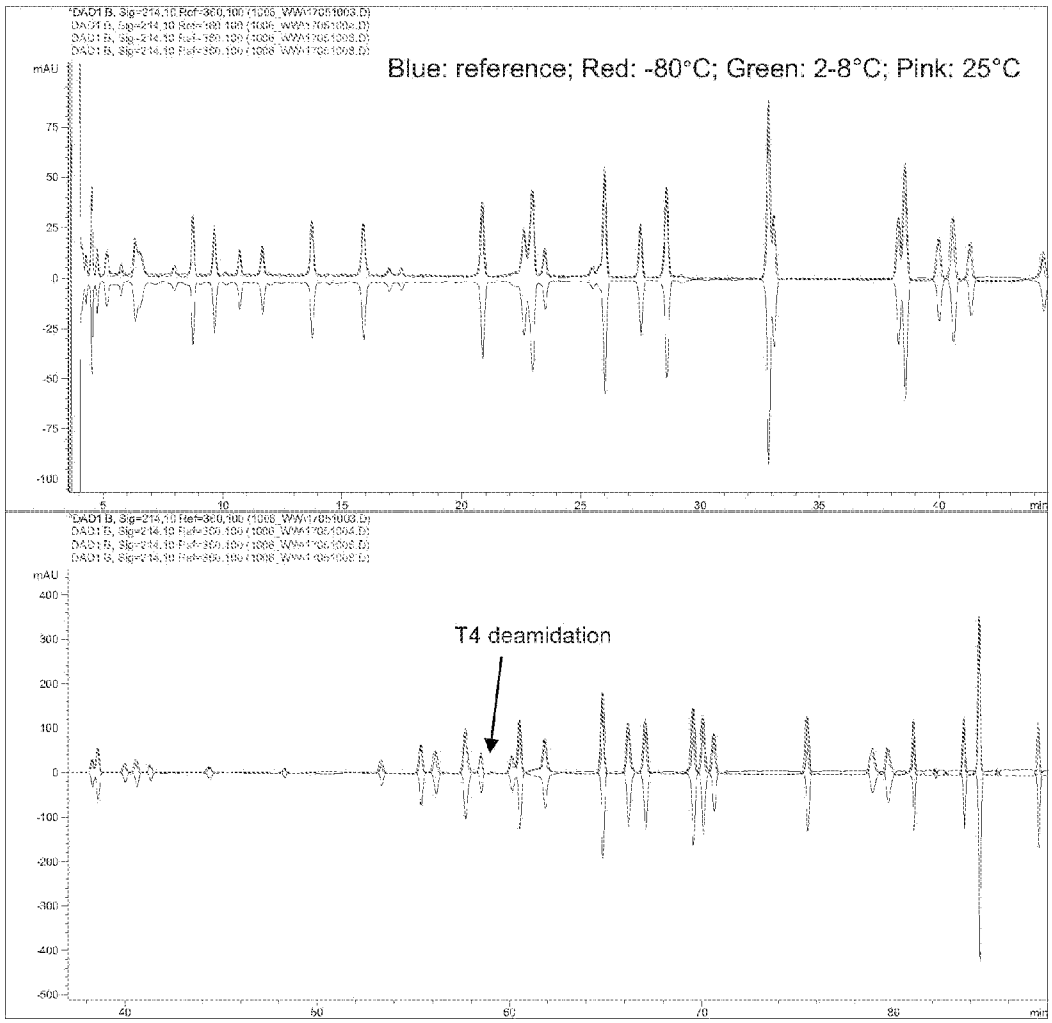

Blue: reference; Red: -80°C; Green: 2-8°C; Pink: 25°C
mAU
min
T4 deamidation

Figure 11

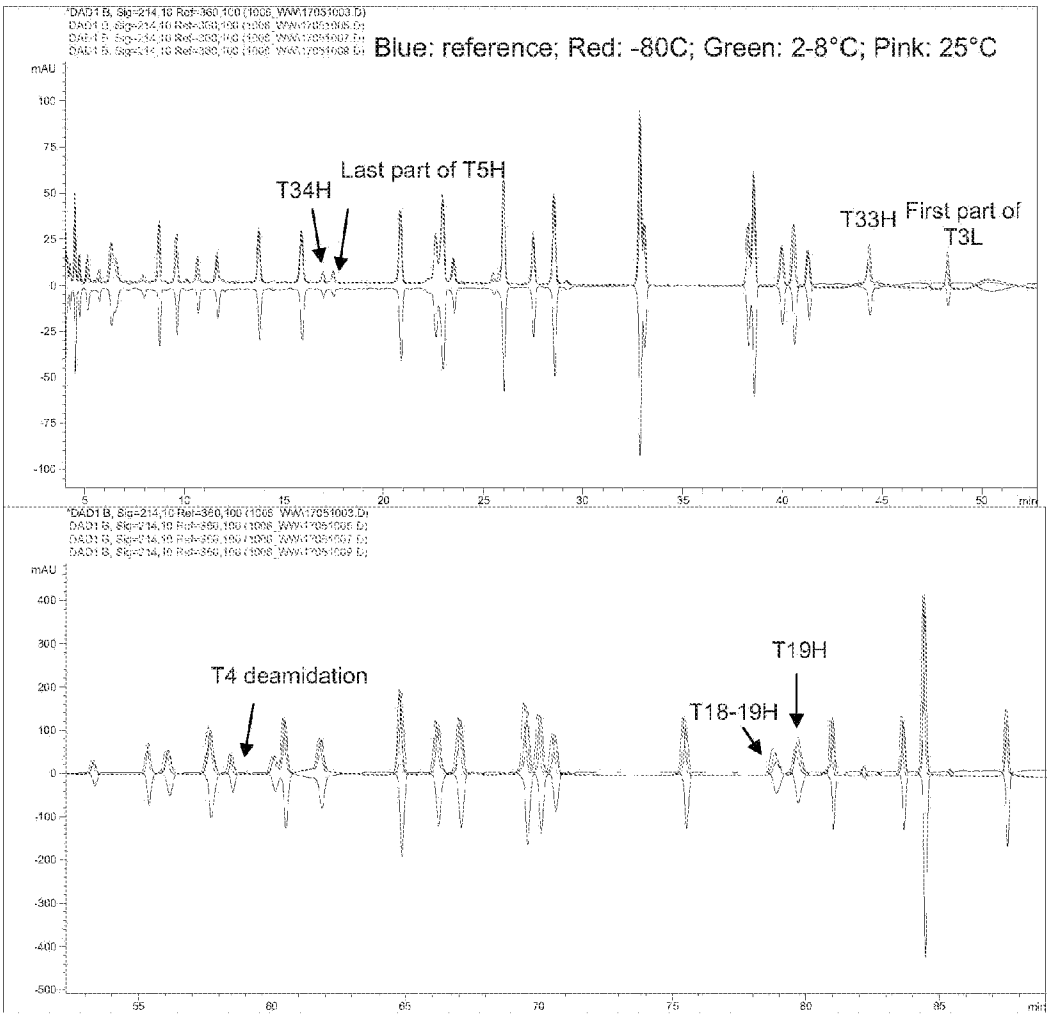

Blue: reference; Red: -80C; Green: 2-8°C; Pink: 25°C
T34H
Last part of T5H
T33H
First part of T3L
T4 deamidation
T18-19H
T19H

Figure 12

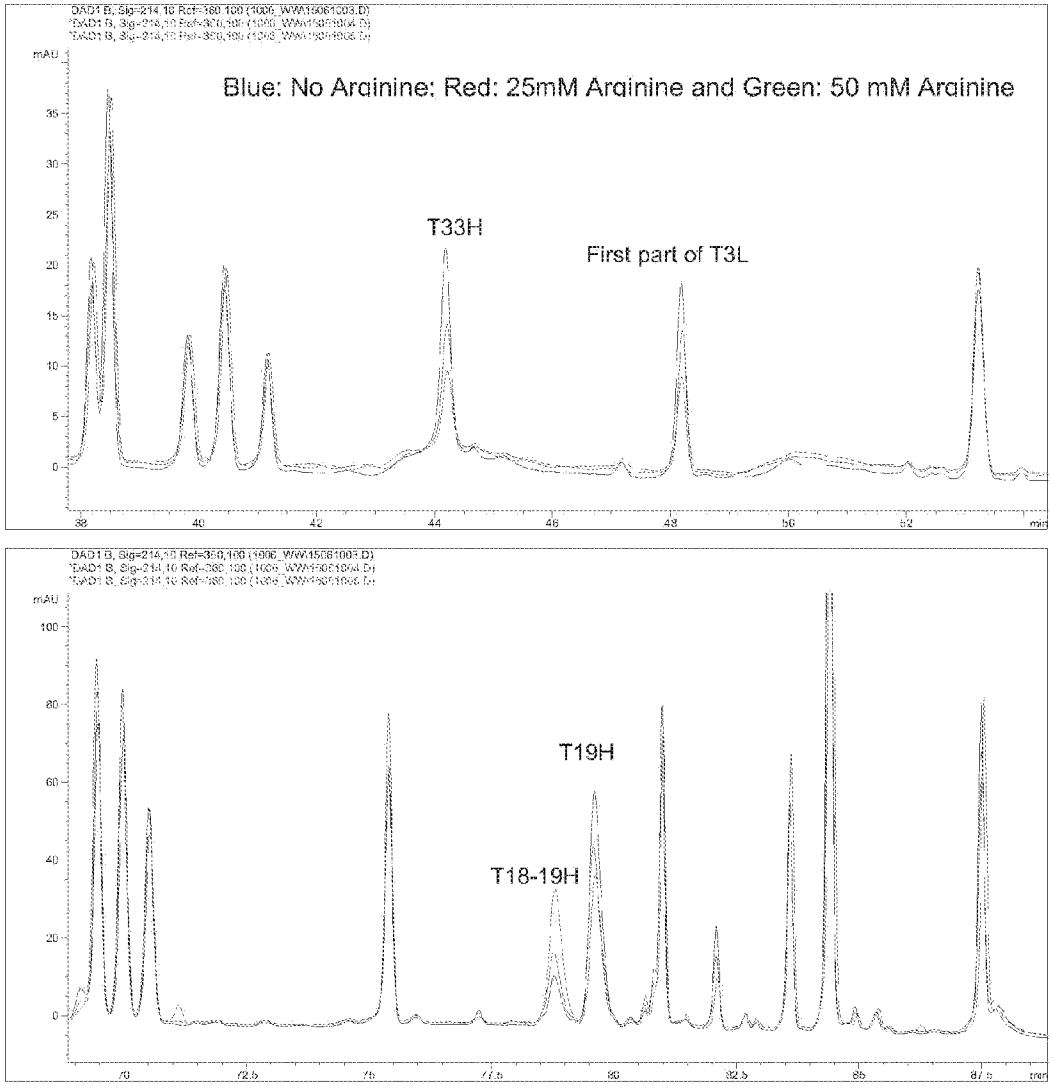

Blue: No Arginine: Red: 25mM Arginine and Green: 50 mM Arginine
T33H
First part of T3L
T19H
T18-19H

Figure 13

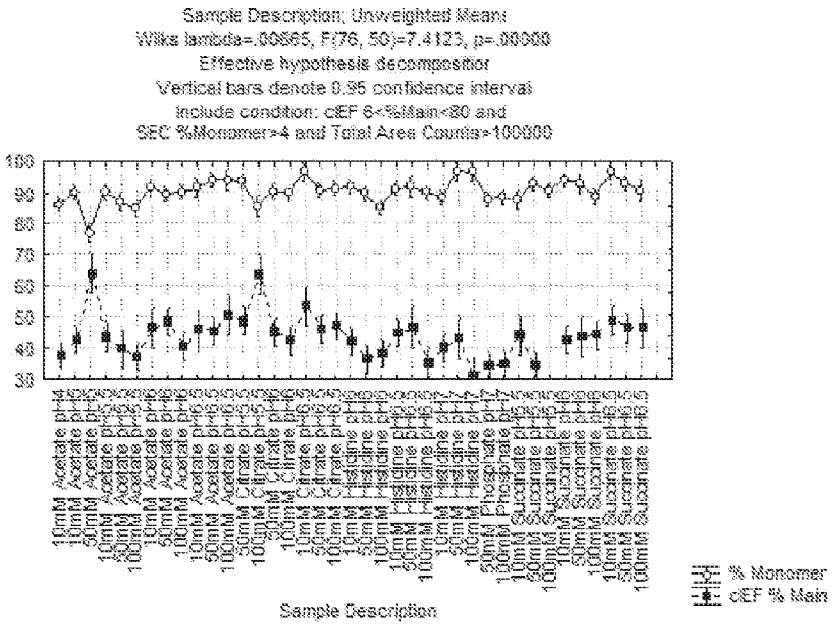

Sample Description; Unweighted Means
Wilks lambda=.00565, F(76, 50)=7.4123, p=.00000
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals
Include condition: cEF 6<%Main<80 and
SEC %Monomer>4 and Total Area Counts>100000
100
90
80
70
60
50
40
30
10mM Acetate pH4
10mM Acetate pH5
50mM Acetate pH5
10mM Acetate pH5.5
50mM Acetate pH5.5
100mM Acetate pH5.5
10mM Acetate pH6
50mM Acetate pH6
100mM Acetate pH6
10mM Acetate pH6.5
50mM Acetate pH6.5
100mM Acetate pH6.5
10mM Citrate pH5
50mM Citrate pH5
100mM Citrate pH5
10mM Citrate pH6
50mM Citrate pH6
100mM Citrate pH6
10mM Histidine pH5.5
50mM Histidine pH5.5
100mM Histidine pH5.5
10mM Histidine pH6
50mM Histidine pH6
100mM Histidine pH6
10mM Histidine pH6.5
50mM Histidine pH6.5
100mM Histidine pH6.5
10mM Phosphate pH6
50mM Phosphate pH6
100mM Phosphate pH6
10mM Succinate pH5
50mM Succinate pH5
100mM Succinate pH5
10mM Succinate pH5.5
50mM Succinate pH5.5
100mM Succinate pH5.5
Sample Description
% Monomer
cEF % Main

Figure 14

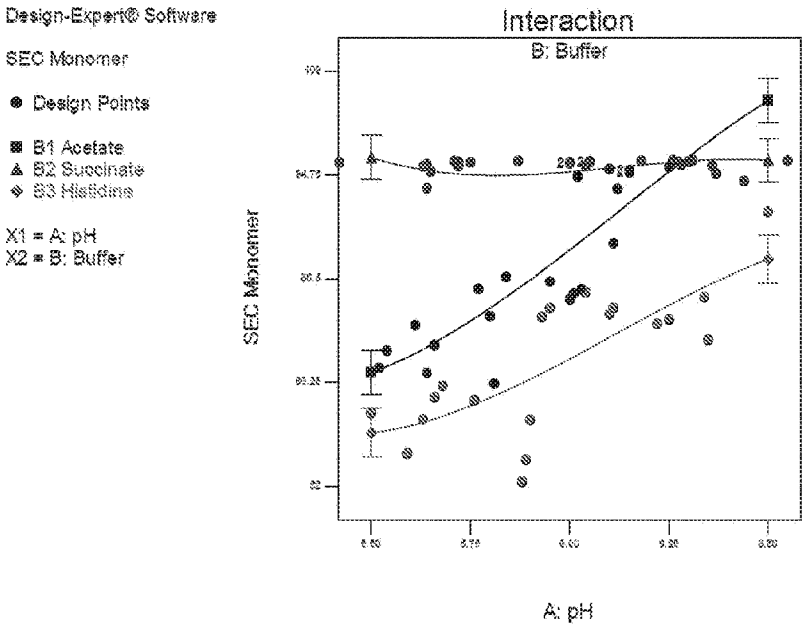

Design-Expert® Software
SEC Monomer
Design Points
B1 Acetate
B2 Succinate
B3 Histidine
X1 = A: pH
X2 = B: Buffer
Interaction
B: Buffer
SEC Monomer
A: pH

Figure 15

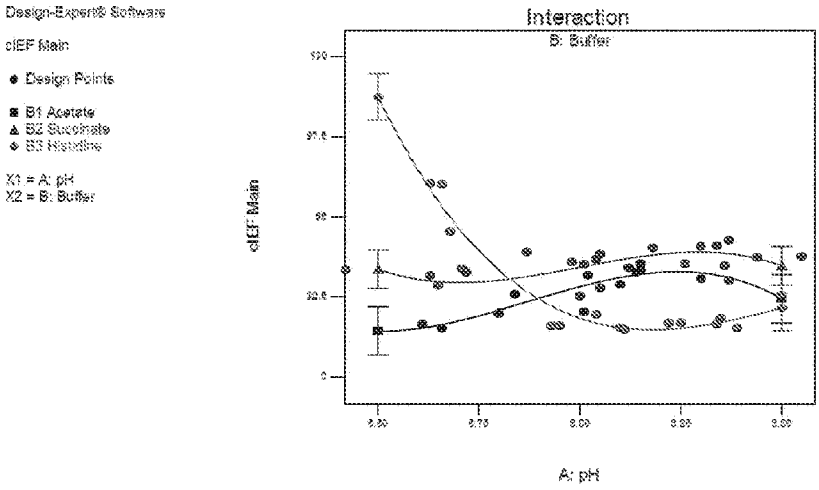

Design-Expert® Software
cIEF Main
Design Points
B1 Acetate
B2 Succinate
B3 Histidine
X1 = A: pH
X2 = B: Buffer
Interaction
B: Buffer
cIEF Main
A: pH

Figure 16

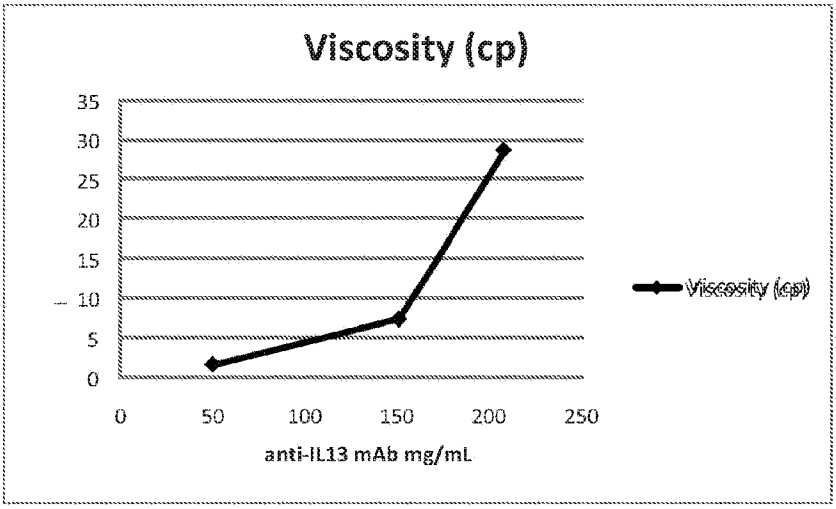
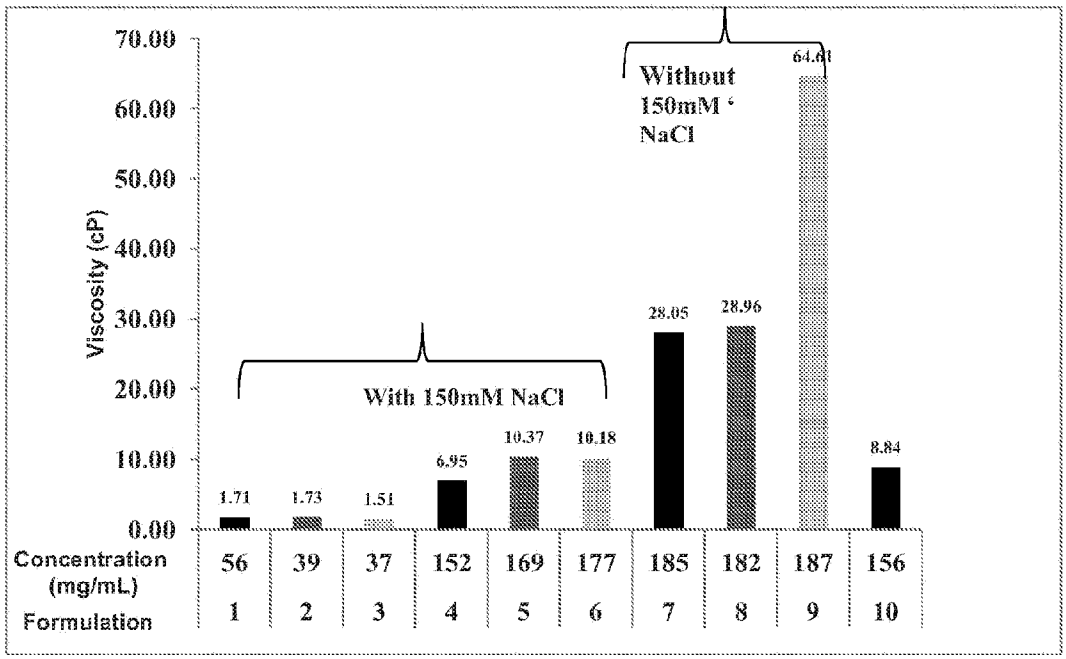

Viscosity (cp)
35
30
25
20
15
10
5
0
0
50
100
150
200
250
anti-IL13 mAb mg/mL
Viscosity (cp)

Figure 17



Black- acetate
Grey- succinate
Light Grey- histidine

Figure 18

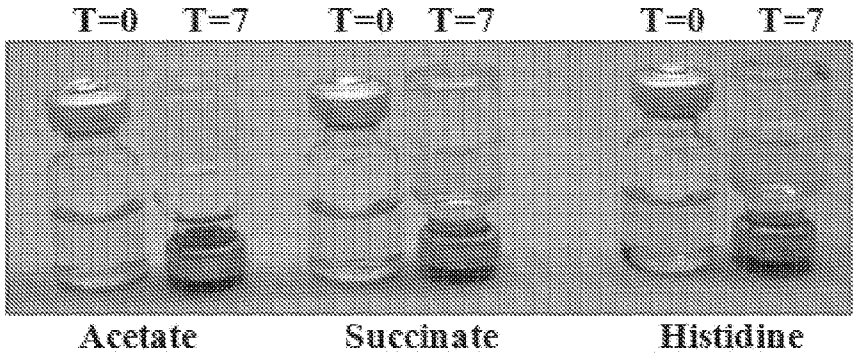


Figure 19

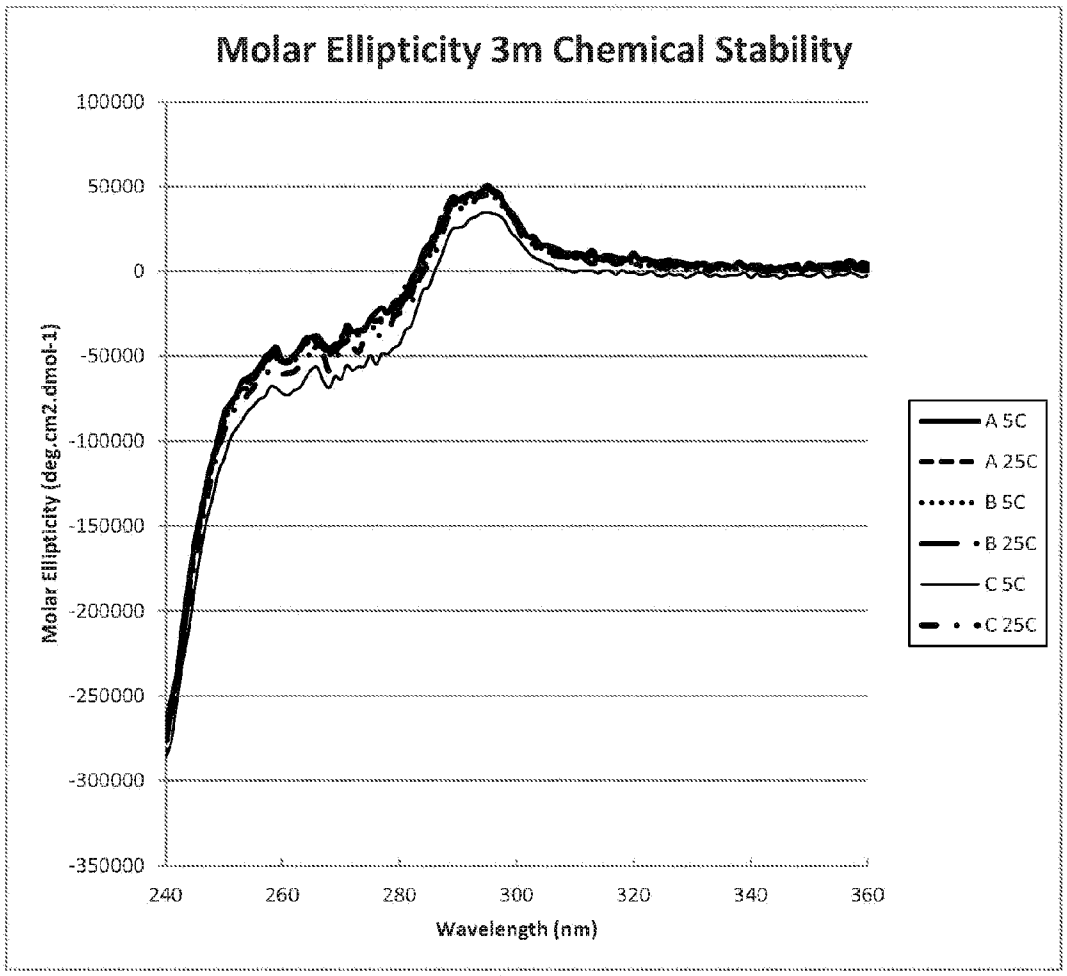

Molar Ellipticity 3m Chemical Stability
100000
50000
0
-50000
-100000
-150000
-200000
-250000
-300000
-350000
Molar Ellipticity (deg.cm2.dmol-1)
240
260
280
300
320
340
360
Wavelength (nm)
A 5C
A 25C
B 5C
B 25C
C 5C
C 25C

Figure 20