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(54) Title: FORMULATIONS OF MONOCLONAL ANTIBODIES

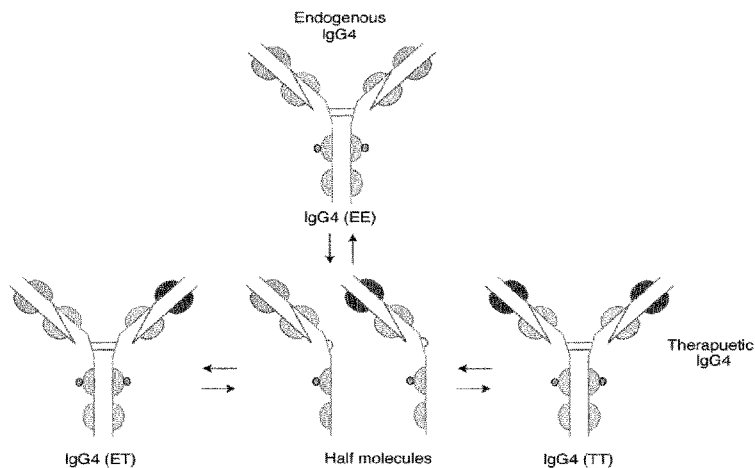


FIG. 1

(57) Abstract: The present invention provides a formulation comprising: (i) a monoclonal antibody; and (ii) an ionic excipient; wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (e.g. about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 6.5.



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## FORMULATIONS OF MONOCLONAL ANTIBODIES

### FIELD OF THE INVENTION

[0001] The invention is concerned with an antibody formulation, in particular, a monoclonal antibody formulation and uses thereof. The invention is particularly concerned with providing improved colloidal stability in an antibody formulation.

### BACKGROUND OF THE INVENTION

[0002] As a result of the isoelectric point (pI) of a number of monoclonal antibodies being in the preferred pharmaceutical pH formulation range for proteins (pH 5.5 to pH 7.5), these molecules present unique formulation challenges.

[0003] Colloidal instability at a molecule's pI is due to a lack of an electrostatic charge on the molecule, which allows closer protein-protein interactions (so-called "self-association") that lead to physical instabilities. For this reason, the pH of a protein formulation is typically selected to be at least 1 pH unit away from the protein pI. This aims to provide colloidal stability and thus prevent physical instabilities, such as aggregation, precipitation, opalescence, phase separation and/or particle formation.

[0004] According to the '1 pH unit away' rule, antibodies having a low or neutral pI *e.g.* a pI of pH 5.5 to pH 7.5 thus should be formulated into a formulation with a pH outside the range of 5.5 to 7.5. However, outside this range, additional instabilities can be observed. At more acidic pH, an increased rate of fragmentation, reduced conformational stability and increased aggregation can be observed. At more basic pH, the potential for increased oxidation, deamidation and fragmentation and incompatibility with glass containers are present.

[0005] The above instabilities are particularly problematic in such antibody formulations where the antibody is present at a commercially desirable concentration *e.g.* 50mg/ml and above.

[0006] Therefore, there exists a need to provide an improved formulation for an antibody having a low or neutral pI. In particular, there exists a need to provide a stable formulation for an antibody having a low or neutral pI and, particularly such a formulation having a commercially desirable antibody concentration.

## BRIEF SUMMARY OF THE INVENTION

[0007] The present invention provides a new antibody formulation, in particular a new monoclonal antibody formulation. In particular, the present formulation provides a means for improving colloidal stability for antibodies having a low or neutral pI. The present invention thus provides an alternative to the '1 pH away' rule for providing colloidal stability. The present invention thus allows antibodies having a low or neutral pI to be formulated within 1 pH unit of the antibody pI. Thus, the present invention enables such antibodies to be formulated within a pH range of 5.5 to 7.5 and at a commercially useful concentration, whilst substantially avoiding the instabilities associated with more acidic or more basic pHs.

[0008] The invention provides a formulation comprising:

- i. a monoclonal antibody; and
- ii. an ionic excipient;

wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (e.g. about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5.

[0009] The invention thus further provides a formulation comprising:

- i. a monoclonal antibody; and
- ii. an ionic excipient;

wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (e.g. about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5; and wherein the aggregation rate of the monoclonal antibody in the formulation is reduced compared to the aggregation rate of the same antibody in the same formulation but without an ionic excipient.

[0010] The formulations of the present invention are particularly useful for antibodies having a low or neutral pI, for example in the range of pH 5.5 to pH 7.5. The invention provides a formulation comprising:

- i. a monoclonal antibody having a low or neutral pI (e.g. 5.5 to 7.5); and
- ii. an ionic excipient;

wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (e.g. about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 to about 150mM and the formulation has a pH of 5.5 to 7.5.

[0011] The invention thus further provides a formulation comprising:

- i. a monoclonal antibody having a low or neutral pI (e.g. 5.5 to 7.5); and
- ii. an ionic excipient;

wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (e.g. about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5; and wherein the aggregation rate of the monoclonal antibody in the formulation is reduced compared to the aggregation rate of the same antibody formulated in the same formulation but without an ionic excipient.

[0012] In one embodiment, the monoclonal antibody has a pI in the range of 5.5 to 7.5. In one embodiment, the monoclonal antibody has a pI in the range of 6.0 to 7.5. In one embodiment, the monoclonal antibody has a pI in the range of 6.3 to 7.5. In one embodiment, the monoclonal antibody has a pI in the range of 6.4 to 7.5. Without wishing to be bound by theory, a low to neutral pI can occur for proteins where there is either a net balance of oppositely charged (positive amine groups and negative carboxylate groups) amino acid side chains on the protein or different domains have overall opposite charge, within a pH range of 5.5 to 7.5. Again, without wishing to be bound by theory, it is possible that the ionic excipient in the formulation of the invention shields these opposing and attractive charges, thus colloidally stabilizing proteins having a pI within this range. The present invention thus provides use of an ionic excipient in an antibody formulation for the purpose of changing the charge state or distribution of the antibody in the formulation. The present invention further provides use of an ionic excipient in an antibody formulation for the purpose of colloidally stabilizing the antibody in the formulation.

[0013] In one embodiment, the monoclonal antibody is present in the formulations described herein at a concentration of about 75 mg/ml or greater (e.g. about 75 mg/ml to about 200 mg/ml). In one embodiment, the monoclonal antibody is present in the formulations described herein at a concentration of about 100 mg/ml or greater (e.g.

about 100 mg/ml to about 200 mg/ml). In one embodiment, the monoclonal antibody is present in the formulations described herein at a concentration of about 100 mg/ml to about 165 mg/ml. In one embodiment, the monoclonal antibody is present at a concentration of about 100mg/ml.

**[0014]** In one embodiment, the ionic excipient is present at a concentration of about 75 mM to about 100 mM. In one embodiment, the ionic excipient is present at a concentration of about 75 mM. In one embodiment, the ionic excipient is present at a concentration of about 80 mM.

**[0015]** In one embodiment, the monoclonal antibody is an IgG1 or IgG4 monoclonal antibody. Most preferably, the monoclonal antibody is an IgG4 monoclonal antibody. IgG4 antibodies typically have a low or neutral pI. The invention thus provides a formulation comprising:

- i. an IgG4 monoclonal antibody; and
- ii. an ionic excipient;

wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (e.g. about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5.

**[0016]** The invention thus further provides a formulation comprising:

- i. an IgG4 monoclonal antibody; and
- ii. an ionic excipient;

wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (e.g. about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5; and wherein the aggregation rate of the monoclonal antibody in the formulation is reduced compared to the aggregation rate of the same antibody in the same formulation but without an ionic excipient.

**[0017]** In one embodiment, the formulations described herein have a pH in the range of about pH 5.5 to about pH 6.5. In one embodiment, the formulations described herein have a pH in the range of about pH 5.7 to about pH 6.3. In one embodiment, the formulations described herein have a pH in the range of about pH 5.7 to about pH 6.1. Preferred

formulations have a pH of about 5.8. Other preferred formulations have a pH of about 6.0.

[0018] In one embodiment, the ionic excipient is a charged amino acid. In one embodiment, the ionic excipient is lysine. In another embodiment, the ionic excipient is arginine.

[0019] In one embodiment, the ionic excipient is a salt. The invention thus provides a formulation comprising:

- i. a monoclonal antibody as defined anywhere herein; and
- ii. a salt;

wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (e.g. about 50 mg/ml to about 200 mg/ml) and the salt is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5.

[0020] The invention thus further provides a formulation comprising:

- i. a monoclonal antibody as defined anywhere herein; and
- ii. a salt;

wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (e.g. about 50 mg/ml to about 200 mg/ml) and the salt is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5; and wherein the aggregation rate of the monoclonal antibody in the formulation is reduced compared to the aggregation rate of the same antibody in the same formulation but without a salt.

[0021] In one embodiment, the salt is present at a concentration of about 75 mM to about 100 mM. In one embodiment, the salt is present at a concentration of about 75 mM or about 80 mM.

[0022] In one embodiment, the salt is NaCl, for example at a concentration of about 75 mM to about 100 mM, suitably at a concentration of about 75 mM.

[0023] In one embodiment, the salt is arginine hydrochloride, for example at a concentration of about 75 mM to about 100 mM, suitably at a concentration of about 80 mM.

[0024] In one embodiment, the formulation further comprises a sugar. Amongst other known benefits, the presence of a sugar can improve tonicity of the formulation. This is

desirable since preferred formulations are isotonic or near isotonic (for example, having an osmolality between 240 to 500 mOsm/kg. In one embodiment, the ionic excipient is a salt and the formulation further comprises a sugar.

[0025] In one embodiment, the formulation further comprises a sugar and the ionic excipient is present at a concentration in the range of about 75mM to about 150 mM. In one embodiment, the formulation further comprises a sugar and the ionic excipient is present at a concentration in the range of about 75mM to about 100mM. In one embodiment, the formulation further comprises a sugar, which is present at a concentration in the range of about 100mM to 140mM, and the ionic excipient is present at a concentration in the range of about 75mM to 100mM.

[0026] The invention thus provides a formulation comprising:

- i. a monoclonal antibody as defined anywhere herein;
- ii. an ionic excipient (*e.g.* a salt) as defined anywhere herein;
- iii. a sugar as defined anywhere herein; and

wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (*e.g.* about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5. The ionic excipient preferably is present at a concentration in the range of about 75mM to about 150 mM, more preferably about 75mM to about 100 mM.

[0027] The invention thus further provides a formulation comprising:

- i. a monoclonal antibody as defined anywhere herein; and
- ii. an ionic excipient (*e.g.* a salt) as defined anywhere herein;
- iii. a sugar as defined anywhere herein; and

wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (*e.g.* about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 of about 150 mM and the formulation has a pH of 5.5 to 7.5; and wherein the aggregation rate of the monoclonal antibody in the formulation is reduced compared to the aggregation rate of the same antibody in the same formulation but without an ionic excipient. The ionic excipient preferably is present at a concentration in the range of about 75mM to about 150 mM, more preferably about 75mM to about 100 mM.



[0028] In one embodiment, the sugar is trehalose. In another embodiment, the sugar is sucrose. For example, the sugar is found at a concentration of about 100 mM to about 140 mM, suitably at a concentration of about 120 mM.

[0029] In one embodiment, the formulation further comprises one or more buffers. In one embodiment, the one or more buffers is a buffer comprising histidine. In one embodiment, the one or more buffers are selected from a buffer comprising histidine succinate, histidine acetate, histidine citrate, histidine chloride or histidine sulfate. In one embodiment, the one or more buffers is histidine, histidine hydrochloride, or a combination thereof (histidine/ histidine hydrochloride). In one embodiment, the one or more buffers is L-histidine/ L-histidine hydrochloride monohydrate. For example, the buffer may be at a concentration of about 10 mM to about 50 mM, suitably at a concentration of about 30 mM. It will be understood that a buffer may, itself, be an ionic excipient. Thus, in one embodiment, the buffer is the ionic excipient. In this embodiment, the concentration of the buffer should be above 50 mM i.e. in line with the concentration of the ionic excipient disclosed herein. Put another way, in one embodiment, the ionic excipient also acts as a buffer in the formulation. In this embodiment, an additional buffer may or may not be present.

[0030] In one embodiment, the formulation further comprises a surfactant. In one embodiment, the surfactant is a polysorbate, including for example, polysorbate-80.

[0031] In one embodiment, the formulation further comprises a sugar and one or more buffers. In one embodiment, the ionic excipient is a salt and the formulation further comprises a sugar and one or more buffers.

[0032] In one embodiment, the formulation further comprises a surfactant, a sugar and one or more buffers. In one embodiment, the ionic excipient is a salt and the formulation further comprises a surfactant, a sugar and one or more buffers.

[0033] The invention thus provides a formulation comprising:

- i. a monoclonal antibody as defined anywhere herein;
- ii. an ionic excipient (*e.g.* a salt) as defined anywhere herein;
- iii. a sugar as defined anywhere herein;
- iv. one or more buffers as defined anywhere herein; and
- v. optionally a surfactant as defined anywhere herein

wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (e.g. about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5.

- [0034] The invention thus further provides a formulation comprising:
- i. a monoclonal antibody as defined anywhere herein; and
  - ii. an ionic excipient (e.g. a salt) as defined anywhere herein;
  - iii. a sugar as defined anywhere herein;
  - iv. one or more buffers as defined anywhere herein; and
  - v. optionally a surfactant as defined anywhere herein

wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (e.g. about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5; and wherein the aggregation rate of the monoclonal antibody in the formulation is reduced compared to the aggregation rate of the same antibody in the same formulation but without an ionic excipient..

- [0035] The formulations described herein can also include one or more additional excipients, including for example, one or more sugars, salts, amino acids, polyols, chelating agents, emulsifiers and/or preservatives.

- [0036] One preferred formulation provided by the invention comprises 150mg/ml antibody, 50mM sodium acetate/acetic acid, 106mM trehalose dehydrate, 70mM sodium chloride, 0.05% (w/v) Polysorbate 80, wherein the formulation has a pH of pH 5.8. The antibody in this formulation is preferably an anti-GM-CSF-R $\alpha$  IgG4 antibody, more preferably an anti-GM-CSF-R $\alpha$  IgG4 antibody having the VH and VL sequences of the CAM-3001 antibody as described herein. The antibody in this formulation is preferably an anti-GM-CSF-R $\alpha$  IgG4 antibody having the same 6 CDRs as the CAM-3001 antibody as described herein.

- [0037] One preferred formulation provided by the invention comprises 150mg/ml antibody, 50 mM Sodium Acetate, 85 mM sodium chloride and 0.01% Polysorbate 80, wherein the formulation has a pH of pH 5.5. The antibody in this formulation is preferably an anti-IL-13 IgG4 antibody, more preferably an anti-IL-13 IgG4 antibody having the VH and VL sequences of the CAT-354 antibody as described herein. The

antibody in this formulation is preferably an anti-IL-13 IgG4 antibody having the same 6 CDRs as the CAT-354 antibody as described herein.

[0038] The formulations of the invention preferably are pharmaceutical formulations.

[0039] The present invention provides a pharmaceutical formulation as described anywhere herein for use as a medicament.

[0040] The present invention provides a pharmaceutical formulation as described anywhere herein for use in the treatment of a disease.

[0041] The present invention provides a method of treating a disease in a subject comprising administering a pharmaceutical formulation as described anywhere herein to the subject. Also provided herein are methods of treating a subject by administering a therapeutically effective amount of a pharmaceutical formulation as described anywhere herein to the subject.

[0042] In one embodiment, the subject is a human.

[0043] The disease to be treated will be dependent on the particular antibody contained in the formulation. In one embodiment, the disease is cancer. The disease may be selected from the group consisting of diabetes, cardiovascular diseases, infectious disease, rheumatoid arthritis, vasculitis, giant cell arthritis, glomerular nephropathy, lupus nephritis, uveitis, atopic dermatitis, cirrhosis, psoriatic arthritis, chronic obstructive pulmonary disease, severe asthma, neutrophilic asthma, and myeloid leukemia.

[0044] The present invention provides a lyophilized cake capable of being reconstituted using only sterile water into a formulation as defined herein or a pharmaceutical formulation as defined herein. Also provided herein is a formulation capable of being lyophilised to form a lyophilized cake, wherein the lyophilized cake is capable of being reconstituted using only sterile water into a formulation as defined herein or a pharmaceutical formulation as defined herein.

#### BRIEF DESCRIPTIONS OF THE DRAWINGS

[0045] FIG. 1 shows the conversion of an IgG4 monoclonal antibody to a half molecule.

[0046] FIG. 1-1 and FIG. 1-2 show sequences relating to the CAT-354 antibody. The  $V_H$  and  $V_L$  sequences are labelled and the 6 CDRs are underlined.

[0047] FIG. 2 shows the effect of salt on the aggregation rate of IgG4 molecules.

- [0048] FIG. 3A shows the results of varying pH on  $k_D$ .
- [0049] FIG. 3B shows the results of varying pH on  $K_d$  in the presence of 100 mM salt.
- [0050] FIG. 4 shows the effect of salt on  $k_D$ . Note generally a reduction in colloidal stability at pH 5, but an increase in colloidal stability at pH 6 and pH 7.
- [0051] FIG. 5 shows aggregation rate data at 40°C for formulations with or without 100 mM salt.
- [0052] FIG. 6 shows change in aggregation rate with formulation pH for MEDI7814.
- [0053] FIG. 7 shows the impact of ionic excipients on antibody aggregation rate at pH6 (MEDI7814).
- [0054] FIG. 8 shows the MEDI8897 heavy chain nucleotide sequence and translation. CDRs are underlined, amino acid differences from allelic constant regions have been circled and division between the variable and constant regions marked by a '¶'.
- [0055] FIG. 9 shows the MEDI8897 light chain nucleotide sequence and translation. CDRs are underlined and division between the variable and constant regions marked by a '¶'.
- [0056] FIG. 10 shows MEDI8897 formulation stability over 3 month period at 5°C, 25°C and 40°C.
- [0057] FIG. 11 shows the impact of ionic excipients on antibody aggregation rate at pH 6 (MEDI578).
- [0058] FIG. 12 shows purity by size-exclusion chromatography.
- [0059] FIG. 13 shows a reduction in subvisible particles in formulations containing ionic excipients under thermal stress conditions. Colloidal stability is improved.

#### DETAILED DESCRIPTION OF THE INVENTION

- [0060] Due to the fact that a number of monoclonal antibodies, and in particular IgG4 antibodies, possess a pI that is close to physiologic pH, *i.e.* the pH generally desired for human administration, difficulties in formulating these monoclonal antibodies occur. These difficulties can relate particularly to colloidal stability.
- [0061] IgG4 monoclonal antibodies are characterized by a structure which prevents complement activation, and also allows for an *in vivo* half molecular exchange (one heavy and one light chain) between two IgG4 molecules, generating a new IgG4 with a

bivalent reactivity. *See* FIG. 1. The IgG4 hinge is three amino acids shorter than the hinge of IgG1, and IgG4 has two cysteines that are available for the covalent interaction between the H-chains. [Aalberse and Schuurman, "IgG4 breaking the rules," *Immunology* 2002 105:9-19].

**[0062]** The present invention provides a new monoclonal antibody formulation, in particular a new IgG4 monoclonal antibody formulation and a new IgG1 monoclonal antibody formulation.

**[0063]** The invention provides a formulation comprising: (i) a monoclonal antibody; and (ii) an ionic excipient (*e.g.* a salt); wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (*e.g.* about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5.

**[0064]** The invention further provides a formulation comprising: (i) a monoclonal antibody; and (ii) an ionic excipient (*e.g.* a salt); wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (*e.g.* about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5; and wherein the aggregation rate of the monoclonal antibody in the formulation is reduced compared to the aggregation rate of the same antibody in the same formulation but without an ionic excipient.

**[0065]** Aggregation rate can be measured according to standard techniques as described herein. Surprisingly, formulations in accordance with the present invention have been shown to have good stability and to have decreased self-aggregation *e.g.* to exhibit  $\leq 2.0$  % aggregation when stored at room temperature for 3 months. The present invention thus provides the use of an ionic excipient in an antibody formulation for the purpose of increasing stability of the antibody in the formulation. The present invention further provides the use of an ionic excipient in an antibody formulation for the purpose of decreasing self-aggregation of the antibody in the formulation.

**[0066]** The formulations of the present invention are particularly useful for antibodies having a low or neutral pI, for example antibodies that have a pI in the range pH 5.5 to pH 7.5, pH 6.0 to pH 7.5, pH 6.3 to pH 7.5, pH 6.4 to pH 7.5, or pH 6.5 to pH 7.5. The pI of an antibody can be measured according to standard techniques, for example by

capillary isoelectric focusing (cIEF). The invention thus provides a formulation comprising: (i) a monoclonal antibody having a low or neutral pI (e.g. 5.5 to 7.5); and (ii) an ionic excipient; wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (e.g. about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5. The invention thus further provides a formulation comprising: (i) a monoclonal antibody having a low or neutral pI (e.g. 5.5 to 7.5); and (ii) an ionic excipient; wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (e.g. about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5; and wherein the aggregation rate of the monoclonal antibody in the formulation is reduced compared to the aggregation rate of the same antibody in the same formulation but without an ionic excipient.

**[0067]** In one embodiment, the monoclonal antibodies have a pI in the range of pH 6.4 to pH 7.5. In another embodiment, the monoclonal antibody described herein has a pI in the range of about pH 5.5 to about pH 6.0, about pH 5.7 to about pH 6.0, or about pH 5.5, about pH 5.6, about pH 5.7, about pH 5.8, about pH 5.9, about pH 6.0, about pH 6.1, about pH 6.2, about pH 6.3, about pH 6.4, or about pH 6.5. In embodiments, the pI of the monoclonal antibodies provided herein is 5.7 to 6.0, more suitably a pI of about 5.8 or 6.0.

**[0068]** In one embodiment, the monoclonal antibody is an IgG1 or IgG4 monoclonal antibody. Most preferably, the monoclonal antibody is an IgG4 monoclonal antibody. The invention thus provides a formulation comprising: (i) an IgG4 monoclonal antibody having a low or neutral pI (e.g. 5.5 to 7.5); and (ii) an ionic excipient; wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (e.g. about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5. The invention thus further provides a formulation comprising: (i) an IgG4 monoclonal antibody having a low or neutral pI (e.g. 5.5 to 7.5); and (ii) an ionic excipient; wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater (e.g. about 50 mg/ml to about 200 mg/ml) and the ionic excipient is present at a concentration of about 50 to

about 150 mM and the formulation has a pH of 5.5 to 7.5; and wherein the aggregation rate of the monoclonal antibody in the formulation is reduced compared to the aggregation rate of the same antibody in the same formulation but without an ionic excipient.

[0069] In one embodiment, the monoclonal antibody is an anti-GM-CSF-R $\alpha$  monoclonal antibody, an anti-IL-13 monoclonal antibody, an anti-RSV monoclonal antibody, or an anti-C5/C5a monoclonal antibody. In exemplary embodiments, the monoclonal antibody is an IgG4 monoclonal antibody, for example, an anti-GM-CSF-R $\alpha$  monoclonal antibody. In one embodiment, the monoclonal antibody is CAM-3001. In one embodiment, the monoclonal antibody is MEDI8897.

[0070] Monoclonal antibodies include antibody functional parts, e.g., antibodies or antigen-binding fragments, variants, or derivatives thereof. Monoclonal antibodies further include, but are not limited to, human, humanized, or chimeric antibodies, single chain antibodies, bispecific antibodies, epitope-binding fragments, e.g., Fab, Fab' and F(ab')<sub>2</sub>, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VL or VH domain, fragments produced by a Fab expression library. ScFv molecules are known in the art and are described, e.g., in US patent 5,892,019. Immunoglobulin or antibody molecules encompassed by this disclosure can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In a preferred embodiment, the monoclonal antibody is an IgG1 or IgG4 monoclonal antibody.

### **Antibody Concentration**

[0071] Suitably, the monoclonal antibody is present in the formulations described herein at a commercially desirable concentration e.g. of about 50 mg/ml to about 300 mg/ml, about 50 mg/ml to about 200 mg/ml, about 100 mg/ml to about 200 mg/ml, about 100 mg/ml to about 165 mg/ml, about 100 mg/ml to about 150 mg/ml, or about 50 mg/ml, about 75 mg/ml, about 100 mg/ml, about 105 mg/ml, about 110 mg/ml, about 115 mg/ml, about 120 mg/ml, about 125 mg/ml, about 130 mg/ml, about 135 mg/ml, about 140 mg/ml, about 145 mg/ml, about 150 mg/ml, about 155 mg/ml, about 160 mg/ml, about 165 mg/ml, about 170 mg/ml, about 175 mg/ml, about 180 mg/ml, about 185 mg/ml,

about 190 mg/ml, about 195 mg/ml, or about 200 mg/ml, including values and ranges within these ranges.

[0072] Suitably, the monoclonal antibody is present in the formulations described herein at a concentration of about 100 mg/ml to about 165 mg/ml. Suitably, the monoclonal antibody is present in the formulations described herein at a concentration of about 100 mg/ml.

### **pH**

[0073] Suitably, the formulations described herein have a pH in the range of about pH 5.5 to about pH 7.5 in order to provide near optimal or optimal chemical stability (hydrolysis, deamidation, isomerization). In one embodiment, the formulations described herein have a pH in the range of about pH 5.7 to about pH 6.3. In one embodiment, the formulations described herein have a pH in the range of about 5.5 to about 6.5. In one embodiment, the formulations described herein have a pH in the range of about pH 5.7 to about pH 6.1. Preferred formulations have a pH of about 5.8. Other preferred formulations have a pH of about 6.0.

[0074] Suitably, the formulations described herein have a pH in the range of about pH 5.5 to about pH 6.0, about pH 5.7 to about pH 6.0, or about pH 5.5, about pH 5.6, about pH 5.7, about pH 5.8, about pH 5.9, about pH 6.0, about pH 6.1, about pH 6.2, about pH 6.3, about pH 6.4, or about pH 6.5. In embodiments, the pH of the formulations provided herein is 5.7 to 6.0, more suitably the formulations have a pH of about 5.8 or 6.0.

[0075] A formulation pH close to about pH 7.4 also can be desirable for injection site tolerability.

### **Ionic Excipient**

[0076] Exemplary ionic excipients for use in the formulations include salts and charged amino acids. The ionic excipient might comprise a combination of a salt and charged amino acid.

[0077] Exemplary charged amino acids include arginine and lysine.



- [0078] Exemplary salts include salts of charged amino acids, for example, succinate, acetate, and sulfate salts of arginine and lysine.
- [0079] Further exemplary salts are those described herein including, but not limited to, sodium chloride, as well as other salts with sodium, potassium, calcium, magnesium and the like, such as chlorides, carbonates, sulphates, acetates, gluconates, lactates, malates, and other auxiliaries and the like which are customary in the field of parenteral administration.
- [0080] Suitably the salt is selected from sodium chloride (NaCl), lysine hydrochloride and arginine hydrochloride. In one embodiment, the salt is NaCl. In another embodiment, the salt is arginine hydrochloride. In another embodiment, the salt is lysine hydrochloride.
- [0081] The concentration of the ionic excipient, suitably salt, in the pharmaceutical formulations described herein is generally in the range of about 50 mM to about 150 mM, more suitably about 50 mM to about 100 mM, about 60 mM to about 80 mM, or about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM or about 100 mM, including any ranges or values within these ranges.
- [0082] In one embodiment, the ionic excipient is present at a concentration of about 50 mM to about 125 mM.
- [0083] In one embodiment, the ionic excipient is present at a concentration of about 50 mM to about 100 mM.
- [0084] In one embodiment, the ionic excipient is present at a concentration of about 75 mM to about 100 mM.
- [0085] In suitable embodiments, the salt is NaCl, for example at a concentration of about 50 mM to about 100 mM, suitably at a concentration of about 70 mM.
- [0086] In suitable embodiments, the salt is arginine hydrochloride, for example at a concentration of about 50 mM to about 100 mM, suitably at a concentration of about 80 mM.

**Buffers**

[0087] The formulations described herein suitably comprise one or more buffers. As used herein, “buffer” refers to an excipient for maintaining the pH of a formulation. Exemplary buffers for use in the formulations provided herein include, but are not limited to histidine, histidine hydrochloride (histidine HCl), sodium succinate, sodium acetate, sodium acetate/acetic acid, sodium phosphate, citrate, phosphate, succinate, glycine, and acetate. In one embodiment, the buffer for use in the formulations described herein is sodium acetate/acetic acid. In one embodiment, the one or more buffers is a buffer comprising histidine. In one embodiment, the one or more buffers are selected from a buffer comprising histidine succinate, histidine acetate, histidine citrate, histidine chloride or histidine sulfate. In one embodiment, the one or more buffers is histidine, histidine hydrochloride, or a combination thereof (histidine/ histidine hydrochloride). In one embodiment, the one or more buffers is L-histidine/ L-histidine hydrochloride monohydrate.

[0088] The concentration of a buffer, suitably sodium acetate/acetic acid, in the pharmaceutical formulations described herein is generally in the range of about 10 mM to about 100 mM, more suitably about 15 mM to about 80 mM, about 25 mM to about 75 mM, about 30 mM to about 60 mM, about 40 mM to about 60 mM, about 40 mM to about 50 mM, or about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM or about 75 mM, including any ranges or values within these ranges.

[0089] In one embodiment, the one or more buffers is L-histidine/ L-histidine hydrochloride monohydrate, for example at a concentration of about 10 mM to about 50 mM, suitably at a concentration of about 30 mM.

[0090] The pH of the buffer is preferably in the range of pH 5.5 to pH 6.0.

[0091] It will be understood that a buffer may, itself, be an ionic excipient. Thus, in one embodiment, the buffer is the ionic excipient. In this embodiment, the concentration of the buffer should be above 50 mM i.e. in line with the concentration of the ionic excipient disclosed herein. Preferable concentrations for the buffer in this embodiment are as discussed anywhere herein in relation to the ionic excipient.

[0092] Put another way, in one embodiment, the ionic excipient also acts as a buffer in the formulation. In this embodiment, an additional buffer may or may not be present.

### *Sugars and Surfactants*

[0093] The formulations described herein suitably comprise a sugar, for example, but not limited to, trehalose, lactose, mannitol, mellibiose, melezitose, raffinose, mannotriose, stachyose and sucrose. In other embodiments, a polyol such as trihydric or higher molecular weight sugar alcohols, e.g. glycerin, dextran, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol, can be used. Examples of reducing sugars include, but are not limited to, glucose, maltose, maltulose, iso-maltulose and lactulose. Examples of non-reducing sugars include, but are not limited to, trehalose, non-reducing glycosides of polyhydroxy compounds selected from sugar alcohols and other straight chain polyalcohols. Examples of sugar alcohols include, but are not limited to, monoglycosides, compounds obtained by reduction of disaccharides such as lactose, maltose, lactulose and maltulose. The glycosidic side group can be either glucosidic or galactosidic. Additional examples of sugar alcohols include, but are not limited to, glucitol, maltitol, lactitol and iso-maltulose. In one embodiment, the sugar is selected from the group consisting of trehalose, lactose, mannitol, raffinose and sucrose. In specific embodiments, trehalose is used as a sugar in the formulations described herein. In specific embodiments, sucrose is used as a sugar in the formulations described herein.

[0094] Suitably, the amount of sugar, for example trehalose, in a formulation described herein is about 1 % (w/v) to about 10 % (w/v). Unless otherwise noted, percentage of a component (%) is used herein indicate a weight/volume (w/v) %. In exemplary embodiments, the amount of sugar in a pharmaceutical formulation described herein is about 1 % (w/v) to about 8 % (w/v), or about 2 % (w/v) to about 6 % (w/v), about 2 % (w/v) to about 5 % (w/v), about 3 % (w/v) to about 5 % (w/v), or about 1 % (w/v), about 2 % (w/v), about 3 % (w/v), about 4 % (w/v), about 5 % (w/v), about 6 % (w/v), about 7 % (w/v), about 8 % (w/v), about 9 % (w/v), or about 10 % (w/v), including any values and ranges within these ranges.

[0095] The formulations described herein suitably comprise a surfactant.

**[0096]** The term “surfactant” as used herein refers to organic substances having amphipathic structures; namely, they are composed of groups of opposing solubility tendencies, typically an oil- soluble hydrocarbon chain and a water-soluble ionic group. Surfactants can be classified, depending on the charge of the surface-active moiety, into anionic, cationic, and nonionic surfactants. Surfactants are often used as wetting, emulsifying, solubilizing, and dispersing agents for various pharmaceutical formulations and preparations of biological materials. Pharmaceutically acceptable surfactants like polysorbates (e.g. polysorbates 20, 40, 60 or 80); polyoxamers (e.g. poloxamer 188); Triton; sodium octyl glycoside; lauryl-, myristyl-, linoleyl- , or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (e.g. lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUA™ series (Mona Industries, Inc., Paterson, N. J.), polyethyl glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol (e.g. Pluronics, PF68 etc), can be used in the pharmaceutical formulations described herein. Suitably the surfactant is a polysorbate, including for example, polysorbate-20, polysorbate-40, polysorbate-60, and polysorbate-80. In one embodiment, the surfactant is polysorbate-80.

**[0097]** Suitably, the formulations described herein comprise a surfactant (suitably polysorbate-80) at about 0.001 % to about 0.5 % (w/v), more suitably about 0.002 % to about 0.1 % of a surfactant, for example about 0.01 % to about 0.2 %, about 0.02 % to about 0.01 %, about 0.02 % to about 0.07 %, about 0.03 % to about 0.06 %, about 0.04 % to about 0.06 %, or about 0.02 %, about 0.025 %, about 0.03 %, about 0.035 %, about 0.04 %, about 0.045 %, about 0.05 %, about 0.055 %, about 0.060 %, about 0.065 %, about 0.07 %, about 0.075 %, about 0.08 %, about 0.085 %, about 0.09 %, about 0.095 %, or about 0.1% of a surfactant, including any ranges or values within these ranges.

**[0098]** The formulations described herein suitably comprise a surfactant and a sugar. The formulations described herein suitably comprise a surfactant and one or more buffers. The formulations described herein suitably comprise a sugar and one or more buffers.

The formulations described herein suitably comprise a surfactant, a sugar, and one or more buffers.

[0099] The formulations described herein can also include one or more additional excipients, including for example, one or more sugars, salts, amino acids, polyols, chelating agents, emulsifiers and/or preservatives.

### **Pharmaceutical Use**

[00100] The formulations of the invention preferably are pharmaceutical formulations. Suitably, the pharmaceutical formulations described herein are “pharmaceutically acceptable,” and thus would meet the necessary approval requirements required by a regulatory agency of the Federal or a state government, or listed in the U.S. Pharmacopeia, European Pharmacopeia, or other generally recognized pharmacopeia, so as to be used in animals, and more particularly in humans.

[00101] The present invention provides a pharmaceutical formulation as described anywhere herein for use as a medicament. The present invention provides a pharmaceutical formulation as described anywhere herein for use in the treatment of a disease. The present invention provides a method of treating a disease in a subject comprising administering a pharmaceutical formulation as described anywhere herein to the subject. Also provided herein are methods of treating a subject by administering a therapeutically effective amount of a pharmaceutical formulation as described anywhere herein to the subject.

[00102] As used herein, the term “subject” includes any human or nonhuman animal. The term “nonhuman animal” includes all vertebrates, for example, but not limited to, mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc. In one embodiment, the subject is a human.

[00103] The disease may be selected from the group consisting of diabetes, cardiovascular disease, infectious disease, rheumatoid arthritis, vasculitis, giant cell arthritis, glomerular nephropathy, lupus nephritis, uveitis, atopic dermatitis, cirrhosis, psoriatic arthritis, chronic obstructive pulmonary disease, severe asthma, neutrophilic asthma, and myeloid leukemia.

- [00104] In embodiments, the formulation is administered to a subject subcutaneously or by injection.
- [00105] Suitably, the formulations are a liquid formulation or a frozen formulation.
- [00106] Also provided herein are methods of preparing a pharmaceutical formulation comprising preparing a pharmaceutical formulation as described herein, and suitably loading the pharmaceutical formulation into a syringe to form a pre-filled syringe.
- [00107] Suitably, the pharmaceutical formulations described herein are prepared in sterile water, or are resuspended in sterile water for injection at the desired volume.
- [00108] In exemplary embodiments, the pharmaceutical formulations have a volume of about 0.1 mL to about 20.0 mL, more suitably about 0.5 mL to about 15.0 mL, about 0.5 mL to about 12.0 mL, about 1.0 mL to about 10.0 mL, about 1.0 mL to about 5.0 mL, about 1.0 mL to about 2.0 mL or about 0.5 mL, about 0.6 mL, about 0.7 mL, about 0.8 mL, about 0.9 mL, about 1.0 mL, about 1.1 mL, about 1.2 mL, about 1.3 mL, about 1.4 mL, about 1.5 mL, about 1.6 mL, about 1.7 mL, about 1.8 mL, about 1.9 mL, about 2.0 mL, about 2.1 mL, about 2.2 mL, about 2.3 mL, about 2.4 mL, about 2.5 mL, about 2.6 mL, about 2.7 mL, about 2.8 mL, about 2.9 mL, or about 3.0 mL, including any ranges or values within these ranges.
- [00109] While in suitable embodiments, the pharmaceutical formulations described herein are liquid formulations, i.e., pharmaceutical formulations prepared in sterile water or water for injection (WFI), the pharmaceutical formulations can also be frozen formulations or previously lyophilized formulations.
- [00110] The present invention also provides a lyophilized cake which is capable of being reconstituted using only sterile water into a formulation according to the invention as described herein. It will be understood that the ratio of antibody: ionic excipient will be the same in the lyophilized cake as in the post-lyophilized formulation. In one embodiment, the molar ratio of ionic excipient:antibody is in the range 450:1 to 40:1. Where the formulation has been lyophilized, the concentrations provided herein for the formulation are the post-reconstitution concentrations and thus are the concentrations in the so-called 'drug product'. By way of example, if a half-reconstitution strategy is used (where half the volume of water removed during lyophilization is returned during reconstitution), then after reconstitution, the concentration of the antibody will be twice

what it was prior to lyophilization i.e. twice what is was in the so-called pre-lyophilization ‘drug-substance’ composition. It will therefore be understood that the present invention further provides a composition capable of being lyophilized to form a lyophilized cake, wherein the lyophilized cake is capable of being reconstituted using only sterile water into a formulation according to the invention as described herein. Suitable reconstitution strategies will be known to those skilled in the art.

[00111] In embodiments, it is desirable to prepare frozen formulations by providing a liquid pharmaceutical formulation as described herein, and freezing the formulation under appropriate conditions. For example, the frozen formulations can be provided by freezing the liquid formulations to less than 0 °C, more suitably to about -20 °C, about -40 °C, about -60 °C, or suitably to about -80 °C. The pharmaceutical formulations are also suitably prepared as liquid formulations and stored at about 2 °C to about 8 °C, or about 2 °C, about 3 °C, about 4 °C, about 5 °C, about 6 °C, about 7 °C or about 8 °C.

[00112] Suitable protocols and methods for preparing lyophilized pharmaceutical formulations from liquid and/or frozen formulations are known in the art.

### **Stability of Formulations**

[00113] In exemplary embodiments, the formulations described herein are stable for extended periods of storage at room temperature or at a temperature range of about 2 °C to about 8 °C, suitably about 5 °C. As used herein, room temperature is generally in the range of about 22 °C to about 25 °C. Suitably the pharmaceutical formulations are stable after storage at about 2 °C to about 8 °C (e.g. 5 °C) for at least six (6) months. As used herein, the term “stable” for a period of storage (or “stability”) is used to indicate that the monoclonal antibody, suitably IgG4 monoclonal antibody, pharmaceutical formulations resist aggregation, degradation, half antibody formation, and/or fragmentation. The stability of the monoclonal antibodies can be assessed by degrees of aggregation, degradation, half antibody formation or fragmentation, as measured by high performance size exclusion chromatography (HPSEC), static light scattering (SLS), Fourier Transform Infrared Spectroscopy (FTIR), circular dichroism (CD), urea unfolding techniques, intrinsic tryptophan fluorescence, differential scanning calorimetry, and/or ANS binding techniques, compared to a reference.

- [00114] The overall stability of a pharmaceutical formulation comprising monoclonal antibodies can be assessed by various immunological assays including, for example, ELISA and radioimmunoassay using isolated antigen molecules.
- [00115] The phrase “low to undetectable levels of aggregation” as used herein refers to pharmaceutical formulations containing no more than about 5 %, no more than about 4 %, no more than about 3 %, no more than about 2 %, no more than about 1 %, or no more than about 0.5 % aggregation by weight of protein as measured by high performance size exclusion chromatography (HPSEC) or static light scattering (SLS) techniques. Suitably, the pharmaceutical formulations exhibit  $\leq 5.0$  % aggregation, more suitably  $\leq 4.0$  % aggregation,  $\leq 3.0$  % aggregation,  $\leq 2.0$  % aggregation,  $\leq 1.0$  % aggregation, or 0.5 % aggregation. Suitably, the liquid pharmaceutical formulations and/or frozen pharmaceutical formulations exhibit  $\leq 5.0$  % aggregation, more suitably  $\leq 4.0$  % aggregation,  $\leq 3.0$  % aggregation,  $\leq 2.0$  % aggregation,  $\leq 1.0$  % aggregation, or 0.5 % aggregation.
- [00116] The term “low to undetectable levels of fragmentation” as used herein refers to pharmaceutical formulations containing equal to or more than about 80 %, about 85 %, about 90 %, about 95 %, about 98 %, or about 99 % of the total monoclonal antibody, for example, in a single peak as determined by HPSEC, or reduced Capillary Gel Electrophoresis (rCGE), representing the non-degraded monoclonal antibody, or a non-degraded fragment thereof, and containing no other single peaks having more than about 5 %, more than about 4 %, more than about 3 %, more than about 2 %, more than about 1 %, or more than about 0.5 % of the total monoclonal antibody. Fragmentation may be measured suitably in IgG4 monoclonal antibodies. Without wishing to be bound by theory, it is thought that decreased self-aggregation is due to improved colloidal stability, as evidenced by increased kD value. In exemplary embodiments, the formulations described herein have reduced opalescence and decreased phase separation as detected by visual observation, light scattering, nephelometry or turbidimetric methods.
- [00117] Further embodiments, features, and advantages of the embodiments, as well as the structure and operation of the various embodiments, are described in detail below with reference to accompanying drawings.



## **EXAMPLES**

### **EXAMPLE 1 – IgG4 Formulations**

- [00118] The formulation challenge of antibodies with low or neutral pI values is that when these antibodies are formulated at a pH outside the range of 5.5 to 7.5 (as generally needed to increase protein charge and colloidal stability), additional instabilities are observed. At acidic pH an increased rate of fragmentation, reduced conformational stability and increased aggregation are observed. At basic pH the potential for increased oxidation, deamidation and fragmentation and incompatibility with glass containers are present. IgG4 antibodies are particularly useful for studying such instabilities since IgG4 antibodies typically have low or neutral pI values.
- [00119] The following details the methods used to develop the formulations described herein that have optimized the storage stability of monoclonal antibodies that possess a neutral pI, and also overcomes the neutral pH physical instabilities mentioned above.
- [00120] As described herein, the formulations have a combination of neutral pH and an ionic excipient such as a salt. Optionally a sugar may also be used in the formulations and this has been shown to provide further improvements in some cases.
- [00121] The sugar (sucrose and trehalose are examples) can (in combination with an ionic excipient) further increase conformational stability; and the ionic excipient (sodium chloride, lysine hydrochloride and arginine hydrochloride for example) increases the colloidal stability of the monoclonal antibody molecules. Furthermore, the neutral pH (about pH 6) of the formulations also minimizes the acidic and basic degradation pathways described above. These formulations provide superior overall storage stability for these monoclonal antibodies. Other suitable components of the formulations are a buffer with a neutral pKa (e.g., NaAC, histidine HCl, sodium phosphate).
- [00122] As described herein, and listed in Table 1, CAM-3001 (an anti-GM-CSFR $\alpha$  monoclonal antibody), two anti-IL-13 monoclonal antibodies, and an anti-C5/C5a monoclonal antibody were chosen for this study as they are IgG4's with neutral pI values.

TABLE 1: IgG4 Molecules Studied

Molecule	Type	pI
CAM-3001 (anti-GM-CSF-R $\alpha$ )	IgG4	6.7
anti-IL13 mAb A (CAT-354)	IgG4	7.4
anti-C5/C5a mAb (MEDI7814)	IgG4-P	6.8-7.3
anti-IL-13 mAb B	IgG4-P	6.6





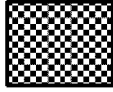














[00123] CAT-354 is a human antibody of the IgG4 subclass that specifically binds the human interleukin 13 (IL-13), blocking interactions with the IL-13 receptor. The DNA and derived amino-acid sequences of the light chain and heavy chain for CAT-354 are provided in **Error! Reference source not found.** and **Error! Reference source not found.**, respectively. In **Error! Reference source not found.** and **Error! Reference source not found.**, the V<sub>H</sub> and V<sub>L</sub> sequences are labelled and the 6 CDRs are underlined. The CAT-354 molecule is a human monoclonal IgG4 (lambda light-chain) antibody with a molecular weight of approximately 147,000 Daltons (Da) (including oligosaccharides). The antibody is composed of two identical heavy chains of approximately 49,500 Da each, and two identical light chains of approximately 22,500 Da each. CAT-354 contains fucosylated biantennary complex and high mannose N-linked carbohydrates attached to each heavy chain at Asn-299. The average size of the oligosaccharide moiety is approximately 1,650 Da per heavy chain.

[00124] CAM-3001 is an antibody which binds to Granulocyte Macrophage Colony Stimulating Factor Receptor alpha (GM-CSFR $\alpha$ ). CAM-3001 is disclosed in International Patent Application Publication WO 2007/110631, the disclosure of which is incorporated by reference herein. The heavy chain CDRs (HCDRs) of CAM-3001 are disclosed as SEQ ID NOs: 53-55 in WO 2007/110631. The light chain CDRs (LCDRs) of CAM-3001 are disclosed as SEQ ID NOs: 58-60 in WO 2007/110631. The heavy chain

variable region (VH) of CAM-3001 is disclosed as SEQ ID NO: 52 in WO 2007/110631. The light chain variable region (VL) of CAM-3001 is disclosed as SEQ ID NO: 218 in WO 2007/110631.

- [00125] As shown in FIG. 2, in the presence of salt at a concentration of 100 mM, the aggregation rate increases when the molecule is at pH 5, but the aggregation rate is reduced when the molecule is at pH 6 and pH 7.
- [00126] The correlation between solution properties of monoclonal antibodies with the diffusion interaction parameter ( $k_D$ ) was examined by testing a combination of conformation stability and colloidal stability in order to achieve the most stable formulation. The  $k_D$  was measured as an indication of colloidal stability, while differential scanning calorimetry (DSC) was examined as a measure of conformational stability. FIG. 3 shows the  $k_D$  at varying pH for the monoclonal antibodies studied. FIG. 3B shows the  $k_D$  at varying pH for the monoclonal antibodies studied after adding salt at a concentration of 100 mM. At low ionic strength, the  $k_D$  generally becomes more negative with increasing pH. The addition of salt makes the  $k_D$  less negative for all molecules studied.
- [00127] In the results depicted in FIG. 4, salt was added to the formulations at a concentration of 100 mM and the colloidal stability examined. Note generally a reduction in colloidal stability at pH 5, but an increase in colloidal stability at pH 6 and pH 7.
- [00128] In the results depicted in FIG. 5, aggregation rate data at 40°C was obtained for formulations with or without 100 mM salt. Formulations with salt near pI (pH 6 or 7) show reduced aggregation compared to formulation without salt away from pI.
- [00129] From the aggregation rate results depicted in FIG. 6 and 7, it can be seen that charged amino acids can also act as ionic stabilizers near the molecule pI and may in some cases be more stabilizing than NaCl.
- [00130] The addition of salt to a histidine-based buffer was examined to determine the effect on the stability of IgG4. The following buffer systems were examined: Control buffer (25 mM histidine, 7 % sucrose, pH 6); Test buffer (25 mM histidine, 7 % sucrose; 100 mM NaCl, pH 6) and the impact of salt on the appearance are summarized in Table 2, below.

Table 2: Comparison and Control and Test Buffer System (Impact of Salt)

	CAM3001	CAT-354	MEDI7814	Anti IL13 NG	
sugar pH 6					 LLPS
sugar pH 7					 Opalescent
sugar, salt, pH 6					
sugar, salt, pH 7					 Clear

LLPS: Liquid-liquid phase separation

[00131] As demonstrated above, the addition of salt reduced the rate of aggregation, and the rate of monomer loss, and improved the appearance of the formulations.

[00132] In summary, formulations comprising salt and with or without sugar have lower opalescence, mitigated phase separation in some instances, and provides as good or better stability when compared to formulations comprising sucrose alone.

[00133] Freeze-thaw studies showed that no significant changes in product quality, such as subvisible particles, were seen after three freeze-thaw cycles. In summary, the addition of both, salt and sugar, improved the stability of the IgG4 molecules, even when formulated at pHs near their pIs.

[00134] **EXAMPLE 2 – IgG1 Formulation**

[00135] MEDI8897 is a human IgG1 $\kappa$ - YTE monoclonal antibody directed against RSV-F protein. Three amino acid substitutions (M252Y/S254T/T256E; called YTE) in the CH2 region of the Fc domain were introduced to increase the serum half-life of MEDI8897. Sequence information for MEDI8897 is provided in Figures 8 and 9. MEDI8897 pI was measured by cIEF to be 6.4-6.7 with the main peak at 6.4. The pI overlaps with the

formulation buffer range (5.5-6.5) suggesting potential issues with manufacturing, formulation and storage stability.

[00136] MEDI8897 thermal stability was measured by differential scanning calorimetry. Tm1 was found to be 61°C while Tm2 was 82°C. A Tm1 greater than 50°C suggests that the molecule has acceptable colloidal stability.

#### Stability Summary

[00137] Upon receipt of MEDI8897 in a standard buffer (25 mM Histidine, 7% sucrose, pH 6.0), phase separation was observed at 2 to 8°C. The supernatant layer had a protein concentration of 75 mg/ml while the bottom layer was 125 mg/ml. Upon equilibration at 25°C the two distinct phases disappeared and only one single phase was observed. The phase separation at 2 to 8°C was thought to be due to the pI of MEDI8897 which is close to the formulation pH of 6.0. A scouting study was initiated to find a more appropriate formulation buffer for MEDI8897 stability assessment, targeting a condition which maintained solubility and prevented phase separation of MEDI8897 at 100 mg/ml.

[00138] Formulating in the standard buffer (25 mM histidine, 7% sucrose) at pH's below 5.9 or above 6.7 mitigated phase separation. Addition of 75 mM NaCl to the standard buffer between pH 5.0 and 6.7 also mitigated phase separation. Finally, acetate and phosphate buffers at pH values away from the pI also mitigated phase separation. Based on these screening studies and previous knowledge of mAbs with pIs within the formulation space, an alternate buffer (25mM His/HisHCl, 75mM NaCl, 4% Sucrose, 0.02% PS80, pH 6.0) was selected for evaluation.

#### kD Studies

[00139] For the first kD screen, all samples were evaluated in 25 mM Histidine pH 5.5 base buffer from 2-10 mg/ml at 25°C. This buffer was chosen in lieu of pH 6.0 because MEDI8897 is more soluble at pH 5.5, facilitating DLS measurements which are sensitive to insoluble particles. Ionic excipients including arginine-HCl, lysine-HCl and NaCl were evaluated at 10, 25, 50, 75 and 100 mM concentrations. In addition, proline, alanine, Na<sub>2</sub>SO<sub>4</sub> and histidine were evaluated at the 100 mM concentration only. Finally, 2, 4, and

6% sucrose were evaluated to determine if sucrose influences protein-protein interactions. All conditions were compared to a buffer control (25 mM Histidine pH 5.5).

**[00140]** The control samples showed distinct protein-protein interactions, with the hydrodynamic radius increasing from 6.2 to 7.8 nm from 2-10 mg/ml. Arginine-HCl, lysine-HCl and NaCl showed reduction of protein-protein interactions (PPI) starting at 25 mM concentrations as evidenced by no increase in hydrodynamic size over the 2-10 mg/ml concentration range. No additional effects were seen between 25 and 100 mM. At 100 mM concentration, proline and alanine showed PPI similar to the control while Na<sub>2</sub>SO<sub>4</sub> and Histidine mitigated PPI. Finally, sucrose concentration showed no impact on PPI. This data illustrates that charged excipients (Arg-HCl, Lys-HCl, Histidine and Na<sub>2</sub>SO<sub>4</sub>) mitigate protein-protein interactions while neutral excipients (sucrose, proline, alanine) do not mitigate PPI. Therefore, addition of ionic excipients at pH 5.5 reduced phase separation at 100 mg/ml.

#### 40°C Stability Evaluation

**[00141]** Based on kD screening, several conditions were selected for 40°C stability evaluation. Table 3 summarizes the formulation conditions and 1 month degradation rates seen at 40°C.

**Table 3- 40°C Stability Rates, Formulation Screen 1- Excipient Screening**

Number	Excipient	Conc (mM)	% Mon/ mo	% Agg/mo	% Frag/mo
1	NaCl	25	-5.9	4.2	1.8
2	NaCl	75	-6.1	4.1	1.9
3	NaCl	95	-5.4	3.5	1.9
4	NaCl	120	-5.4	3.5	1.9
5	Arg-HCl	25	-5.4	3.5	1.8
6	Arg-HCl	75	-4.8	2.8	2.0
7	Arg-HCl	95	-4.5	2.6	1.9
8	Arg-HCl	120	-4.8	2.8	2.0
9	Lys-HCl	25	-5.7	3.9	1.9
10	Lys-HCl	75	-5.0	2.7	2.3
11	Lys-HCl	95	-5.1	3.1	2.0

**Table 3- 40°C Stability Rates, Formulation Screen 1- Excipient Screening**

Number	Excipient	Conc (mM)	% Mon/ mo	% Agg/mo	% Frag/mo
12	Lys-HCl	120	-4.9	2.9	2.0

Base buffer for this study was 25 mM Histidine pH 6.0

[00142] This study illustrates that arginine and lysine are more stabilizing than NaCl. In addition, 75 mM and above appears to stabilize against aggregation. Based on this study, arginine was selected as the most stabilizing lyo-friendly excipient and was moved forward to the next set of studies.

#### Drug Product Stability on Final Lyo Cycle/ Representative Material

[00143] Stability of the drug product was evaluated. Three months of data was collected for the post reconstitution formulation of 100 mg/ml in 30 mM L-histidine/L-histidine hydrochloride monohydrate, 80 mM L-arginine hydrochloride, 120 mM sucrose, 0.04% (w/v) polysorbate 80, pH 6.0. Results are shown in Figure 10. Storage at 2-8°C showed virtually no change during the 3 month period, confirming the suitability of the formulation and lyo cycle for clinical use. These data thus demonstrate that the formulation provides appropriate stability and solubility and is suitable for first time in human clinical use.

**Table 4- Drug Product Stability 3 Month Data Summary**

Temperature	HIAC (≥10 μm)	HIAC (≥25 μm)	Bioassay	Recon Time	VI	KF
2-8°C	216	108	97%	2 min	< STD1	1.3%
25°C	522	90	97%	3 min	< STD1	1.4%
40°C	126	0	90%	3 min	< STD2	1.7%

[00144] **EXAMPLE 3- IgG4 formulation**

[00145] Formulations of antibody MEDI578 were tested at pH6. MEDI578 is an IgG4 monoclonal antibody having a pI of 6.3 to 6.8.

[00146] Results are shown in Figure 11.

**[00147]      EXAMPLE 4- IgG4 formulation**

**[00148]**      All formulations that were tested contained 100mg/ml MEDI578, 20mM histidine pH 6.5 without polysorbate. MEDI578 is an IgG4 monoclonal antibody having a pI of 6.3 to 6.8.

**[00149]**      Table 5 - Formulation information

Formulation #	Excipients	Tg'	Osmo (mOsm/kg)	Opalescence	Particles
1	9% Sucrose	-25.8	387	4	2
2	9% Trehalose	-23.6	341	4	2
3	275mM Mannitol	-28.2	331	5	2
4	125mM Lys/35mM NaCl	-30.1	361	3	1
5	20mM Lys/8% sucrose	-25.7	392	3	1
6	50mM Lys/6% sucrose	-26.4	379	3	1
7	80mM Lys/4% sucrose	-27.3	326	3	1
8	80mM NaCl/4% sucrose	-26	365	3	1
9	80mM Arg/4% sucrose	-24.4	363	3	1
10	150mM Mannitol/4% sucrose	-27.8	379	5	2

**[00150]**      Figure 12 shows purity by size-exclusion chromatography.

**[00151]**      Figure 13 shows a reduction in subvisible particles in formulations containing ionic excipients under thermal stress conditions. Colloidal stability is improved.

**[00152]**      All documents, patents, journal articles and other materials cited in the present application are hereby incorporated by reference.

**[00153]**      Although the present invention has been fully described in conjunction with several embodiments thereof with reference to the accompanying drawings, it is to be understood that various changes and modifications can be apparent to those skilled in the art. Such changes and modifications are to be understood as included within the scope of the present invention as defined by the appended claims, unless they depart there from.



## WHAT IS CLAIMED IS:

1. A formulation comprising:
  - i. a monoclonal antibody; and
  - ii. an ionic excipient;wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater and the ionic excipient is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5.
2. A formulation comprising:
  - i. a monoclonal antibody; and
  - ii. an ionic excipient;wherein the monoclonal antibody is present at a concentration of about 50mg/ml or greater and the ionic excipient is present at a concentration of about 50 to about 150 mM and the formulation has a pH of 5.5 to 7.5; and wherein the aggregation rate of the monoclonal antibody in the formulation is reduced compared to the aggregation rate of the same antibody in the same formulation but without an ionic excipient.
3. A formulation according to claim 1 or 2, wherein the monoclonal antibody has a pI in the range of pH 5.5 to pH 7.5.
4. A formulation according to any one of claims 1 to 3, wherein the monoclonal antibody has a pI in the range of pH 6.0 to pH 7.5.
5. A formulation according to any one of claims 1 to 3, wherein the monoclonal antibody has a pI in the range of pH 6.4 to pH 7.5.
6. A formulation according to any one of claims 1 to 5, wherein the monoclonal antibody is an IgG4 monoclonal antibody.
7. A formulation according to any one of claims 1 to 6, wherein the monoclonal antibody is present in the formulation at a concentration of about 100 mg/ml to about 200 mg/ml.
8. A formulation according to claim 7, wherein the monoclonal antibody is present in the formulation at a concentration of about 100 mg/ml.
9. A formulation according to any one of claims 1 to 8, wherein the formulation has a pH in the range of about pH 5.5 to about pH 6.5.
10. A formulation according to any one of claims 1 to 8, wherein the formulation has a pH in the range of about pH 5.7 to about pH 6.3.

11. A formulation according to any one of claims 1 to 8, wherein the formulation has a pH in the range of about pH 5.7 to about pH 6.1.
12. A formulation according to claim 11, wherein the formulation has a pH of about pH 6.0.
13. A formulation according to any one of claims 1 to 12, wherein the ionic excipient is a salt.
14. A formulation according to claim 13, wherein the salt is NaCl.
15. A formulation according to claim 13, wherein the salt is arginine hydrochloride.
16. A formulation according to claim 13, wherein the salt is lysine hydrochloride.
17. A formulation according to any one of claims 1 to 16, wherein the ionic excipient is present at a concentration of about 75 mM to about 100 mM.
18. A formulation according to claim 17, wherein the ionic excipient is present at a concentration of about 80 mM.
19. A formulation according to any one of claims 1 to 18, wherein the formulation further comprises a sugar.
20. A formulation according to claim 19, wherein the sugar is trehalose.
21. A formulation according to claim 19, wherein the sugar is sucrose.
22. A formulation according to any one of claims 19 to 21, wherein the sugar is present at a concentration of about 100 mM to about 140 mM.
23. A formulation according to claim 22, wherein the sugar is present at a concentration of about 120 mM.
24. A formulation according to any one of claims 1 to 23, wherein the formulation further comprises one or more buffers.
25. A formulation according to claim 24, wherein the one or more buffers is selected from histidine, histidine hydrochloride, and histidine/ histidine hydrochloride.
26. A formulation according to claim 25, wherein the one or more buffers is L-histidine/ L-histidine hydrochloride monohydrate.
27. A formulation according to any one of claims 24 to 26, wherein the one or more buffers is present at a concentration of about 10 mM to about 50 mM.
28. A formulation according to claim 27, wherein the one or more buffers is present at a concentration of about 30 mM.

29. A formulation according to any one of claims 1 to 28, wherein the formulation further comprises a surfactant.
30. A formulation according to claim 29, wherein the surfactant is a polysorbate.
31. A formulation according to claim 30, wherein the surfactant is polysorbate-80.
32. A formulation according to any one of claims 29 to 31, wherein the surfactant is present in the formulation at a concentration from about 0.02 % (w/v) to about 0.07 % (w/v).
33. A formulation according to claim 32, wherein the surfactant is present in the formulation at a concentration of about 0.04% (w/v).
34. A formulation according to any one of claims 1 to 33, wherein the formulation further comprises one or more additional excipients, including for example, one or more sugars, salts, amino acids, polyols, chelating agents, emulsifiers and/or preservatives.
35. A formulation according to claim 1 comprising 150 mg/ml anti-IL-13 IgG4 antibody, 50 mM Sodium Acetate, 85 mM sodium chloride and 0.01% Polysorbate 80, wherein the formulation has a pH of pH 5.5.
36. A formulation according to claim 35, wherein the antibody has the 6 CDR sequences of CAT-354 as shown in Figure 1-1 and Figure 1-2.
37. A formulation according to claim 35, wherein the antibody has the VH and VL sequences of CAT-354 as shown in Figure 1-1 and Figure 1-2.
38. A formulation according to claim 1 comprising 150 mg/ml anti-GM-CSF-R $\alpha$  IgG4 antibody, 50mM sodium acetate/acetic acid, 106mM trehalose dehydrate, 70mM sodium chloride, and 0.05% (w/v) Polysorbate 80, wherein the formulation has a pH of pH 5.8.
39. A formulation according to claim 38, wherein the antibody has the 6 CDR sequences of CAM-3001.
40. A formulation according to claim 38, wherein the antibody has the VH and VL sequences of CAM-3001.
41. A formulation according to any one of claims 1 to 40, which is a pharmaceutical formulation.
42. A pharmaceutical formulation according to claim 41 for use as a medicament.
43. A pharmaceutical formulation according to claim 41 for use in the treatment of a disease.

44. A method of treating a disease in a subject comprising administering a pharmaceutical formulation according to claim 41 to the subject.
45. A lyophilized cake capable of being reconstituted using only sterile water into a formulation as defined in any one of claims 1 to 40 or a pharmaceutical formulation as defined in any one of claims 41-43.
46. A formulation capable of being lyophilized to form a lyophilized cake, wherein the lyophilized cake is capable of being reconstituted using only sterile water into a formulation as defined in any one of claims 1 to 40 or a pharmaceutical formulation as defined in any one of claims 41-43.

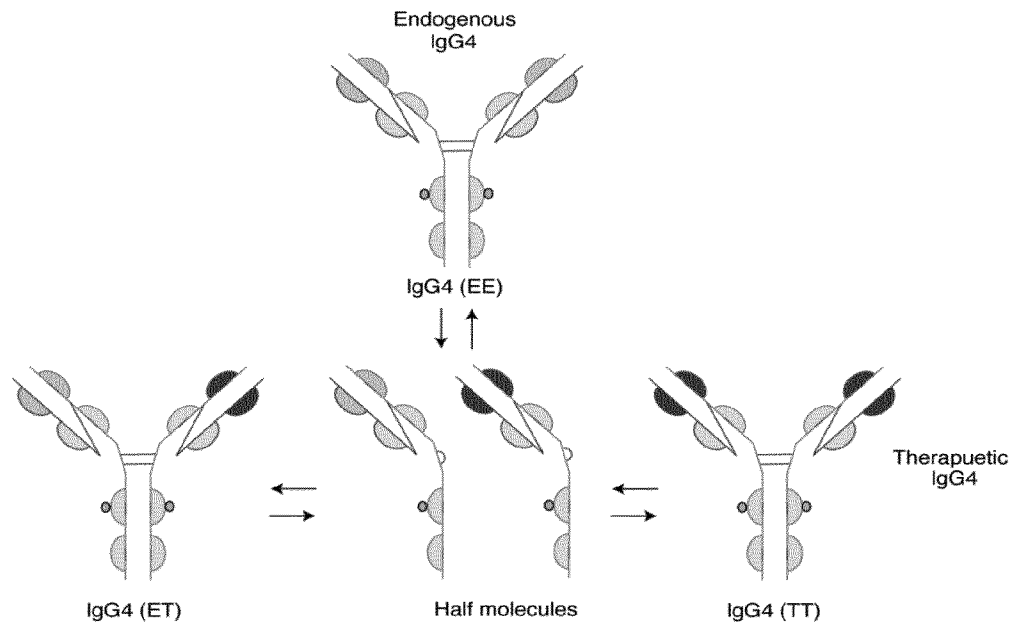


FIG. 1

### DNA and Amino Acid Sequences of CAT-354 Light Chain

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AGTCTAGCGGACCTCTGCGGTAGGTGCGACAAAACCTGGAGGTATCTTCTGTGGCCCTGGC
_____5' UTR_____>

      70      80      90      100     110     120
ATCCAGCCTCCGCGGCCGGGAACGGTGCATTGGAACCGCGGATCCCCGTGCCAAGAGTGA
TAGGTGCGAGGCGCCGGCCCTTGCCACGTAACCTTGCGCCTAAGGGGCACGGTTCTCACT
_____5' UTR_____>

     130     140     150     160           180
CTCACCGTCCTTGACACGAAGCTATTCGAACTACAGTTACTGAGCACACAGGACCTCACC
GAGTGGCAGGAAGTGTGCTTCGATAAGCTTGATGTCAATGACTCGTGTGTCCTGGAGTGG
_____5' UTR_____>

     190     200     210     220     230     240
ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTGTCCACTCCTCC
TACCTACCTCGACATAGTAGGAGAAGAACCATCGTTGTCGATGCCACAGGTGAGGAGG
M G W S C I I L F L V A T A T G V H S>
_____LEADER_____>S>
_____>

     250     260     270     280     290     300
TATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTACC
ATACAGACTGAGTCGGTGGGAGCCACAGTCACCGGGTCCTTCTGCCGGTCTTAATGG
Y V L T Q P P S V S V A P G K T A R I T>
_____CAT-354 VL_____>

     310     320     330     340     350     360
TGTGGGGAAACATCATTGGAAGTAACTTGTACTACTGGTACCAGCAGAAGCCAGGCCAG
ACACCCCTTTGTAGTAACCTTCATTGAAACATGTGACCATGGTCGTCTTCGGTCCGGTC
C G G N I I G S K L V H W Y Q Q K P G Q>
_____CAT-354 VL_____>

     370     380     390     400     410     420
GCCCTGTGCTGGTCATCTATGATGATGGCGACCGGCCCTCAGGGATCCCTGAGCGATTCC
CGGGGACACGACCAGTAGATACTACTACCGCTGGCCGGGAGTCCCTAGGGACTCGCTAAG
A P V L V I Y D D G D R P S G I P E R F>
_____CAT-354 VL_____>

     430     440     450     460     470     480
TCTGGCTCCAACCTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAGGCGGGGAT
AGACCGAGGTTGAGACCCTTGTGCCGGTGGGACTGGTAGTTCGTCCTCCAGCTCCGGCCCCTA
S G S N S G N T A T L T I S R V E A G D>
_____CAT-354 VL_____>

     490     500     510     520     530     540
GAGGCCGACTATTATTGTCAGGTGTGGGATACTGGTAGTGATCCCGTGGTATTCCGGCGGA
CTCCGGCTGATAATAACAGTCCACACCCCTATGACCATCACTAGGGCACCATAAGCCGCT
E A D Y Y C Q V W D T G S D P V V F G G>
_____CAT-354 VL_____>

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      550      560      580      590      600
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G T K L T V L>
_____CAT-354 VL_____>
      G Q P K A A P S V T L F P>
      _____HUMAN C LAMBDA_____>

      610      620      630      640      650      660
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GGGAGGAGACTCCTCGAAGTTCGGTTGTTCCGGTGTGACCACACAGAGTATTCACTGAAG
P S S E E L Q A N K A T L V C L I S D F>
_____HUMAN C LAMBDA_____>

      670      680      690      700      710      720
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ATGGGCCCTCGGCACTGTCACCGGACCTTCCGTCTATCGTCCGGGCAGTTCGCCCTCAC
Y P G A V T V A W K A D S S P V K A G V>
_____HUMAN C LAMBDA_____>

      730      740      750      760      770      780
GAGACCACCACACCCTCCAAACAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGC
CTCTGGTGGTGTGGGAGGTTTGTTCGTTGTTGTTTCATGCGCCGGTTCGTCGATAGACTCG
E T T T P S K Q S N N K Y A A S S Y L S>
_____HUMAN C LAMBDA_____>

      790      800      810      820      830      840
CTGACGCCTGAGCAGTGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGG
GACTGCGGACTCGTCACCTCAGGGTGTCTTCGATGTGACGGTCCAGTGCCTACTTCCC
L T P E Q W K S H R S Y S C Q V T H E G>
_____HUMAN C LAMBDA_____>
                                     >SV40_poly_A_
                                     |
      850      860      870      880      890|      900
AGCACCGTGGAGAAGACAGTGGCCCCCTACAGAATGTTTCATAGGAATTCATTGATCATAAT
TCGTGGCACCTCTTCTGTCACCGGGGATGTCTTACAAGTATCCTTAAGTAACTAGTATTA
S T V E K T V A P T E C S *>
_____HUMAN C LAMBDA_____>

      910      920      930      940      950      960
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GTCGGTATGGTGTAACATCTCCAAAATGAACGAAATTTTTTGGAGGGTGTGGAGGGGGA

      970      980      990      1000      1010      1020
GAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTATTGTCAGCTTATAA
CTTGGACTTTGTATTTTACTTACGTTAACAACAACAATTGAACAAAATAACGTGCAATATT

1030      1040      1050      1060      1070      1080
TGTTACAAATAAAGCAATAGCATCACAATTTTACAAATAAAGCATTTTTTTTC
ACCAATGTTTATTTTCGTTATCGTAGTGTAAAGTGTATTTTCGTAAAAAAG

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FIG. 1-1

### DNA and Amino Acid Sequences of CAT-354 Heavy Chain

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AGTCTAGCGGACCTCTGCGGTAGGTGCGACAAAACCTGGAGGTATCTTCTGTGGCCCTGGC
_____5' UTR_____>

      70      80      90      100     110     120
ATCCAGCCTCCGCGGCCGGGAACGGTGCATTGGAACGCGGATTCCCCGTGCCAAGAGTGA
TAGGTGCGAGGCGCCGGCCCTTGCCACGTAACCTTGCGCCTAAGGGGCACGGTTCTCACT
_____5' UTR_____>

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GAGTGGCAGGAACCTGTGCTTCGAATGACTCGTGTGTCTGGAGTGGTACCCTACCTCGAC
_____M G W S C_____>
_____5' UTR_____>

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ATAGTAGGAGAAGAACCATCGTTGTGATGTCCACAGGTGAGGGTTCACGTCGACCACGT
_____Q V Q L V Q_____>
_____CAT-354VH_____>
  I I L F L V A T A T G V H S>
_____LEADER_____>

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CAGACCCCGACTCCACTTCTTCGGACCCCGGAGTCACTTCCAGAGGACGTTCCGAAGACC
  S G A E V K K P G A S V K V S C K A S G>
_____CAT-354VH_____>

      310     320     330     340     350     360
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AATGTGAAATGTTTAATACCAGAGTCGACCCACGCTGTCCGGGGACCTGTTCCCGAACT
  Y T F T N Y G L S W V R Q A P G Q G L E>
_____CAT-354VH_____>

      370     380     390     400     410     420
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CACCTACCCTACCTAGTCGCGATTATTACCGCTGTGTTAATACCTGTCTTAAGGTCCC
  W M G W I S A N N G D T N Y G Q E F Q G>
_____CAT-354VH_____>

      430     440     450     460     470     480
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_____CAT-354VH_____>

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490 500 510 520 530 540  
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 CTCTAGACTGCTGTGCCGGCAAATAATGACACGCTCTCTGAGGTCGTCGTCGACCCGAGC  
 R S D D T A V Y Y C A R D S S S S W A R >  
 \_\_\_\_\_CAT-354VH\_\_\_\_\_>

550 560 570 580 590 600  
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 GACCAAAAAGCTAGAGACCCCGCCCCCTGTGACCAGTGGCAGAGCTCACGAAGGTGGTT  
 W F F D L W G R G T L V T V S S >  
 \_\_\_\_\_CAT-354VH\_\_\_\_\_>  
 \_\_\_\_\_A S T K>

P30 610 620 630 640 650 660  
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670 680 690 700 710 720  
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 \_\_\_\_\_IG4 CH1\_\_\_\_\_>

790 800 810 820 830 840  
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 \_\_\_\_\_IG4 CH1\_\_\_\_\_>

850 P247 860 870 880 890 900  
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 V D H K P S N T K V D K R V >  
 \_\_\_\_\_IG4 CH1\_\_\_\_\_>

\_\_\_\_\_E S K Y G P>  
 \_\_\_\_\_IGG4 HINGE\_\_\_\_\_>

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 P C P S C P >  
 \_\_\_\_\_IGG4 HINGE\_\_\_\_\_>A P E F L G G P S V F L F P >  
 \_\_\_\_\_IGG4 CH2\_\_\_\_\_>

970 980 990 1000 1010 1020  
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 \_\_\_\_\_IGG4 CH2\_\_\_\_\_>

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 \_\_\_\_\_IGG4 CH2\_\_\_\_\_>

1090 1100 1110 1120 1130 1140  
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 H N A K T K P R E E Q F N S T Y R V V S >  
 \_\_\_\_\_IGG4 CH2\_\_\_\_\_>

1150 1160 1170 1180 1190 1200  
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 \_\_\_\_\_IGG4 CH2\_\_\_\_\_>

1210 1220 1230 1240 1250 1260  
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G Q P R >  
 \_\_\_\_\_>

P41 1270 1280 1290 1300 1310 1320  
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 \_\_\_\_\_IGG4 CH3\_\_\_\_\_>

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 \_\_\_\_\_IGG4 CH3\_\_\_\_\_>

1390 1400 1410 1420 1430 1440  
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 \_\_\_\_\_IGG4 CH3\_\_\_\_\_>

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  F  L  Y  S  R  L  T  V  D  K  S  R  W  Q  E  G  N  V  F  S >
_____IGG4 CH3_____>

1510      1520      1530      1540      1550      1560
ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTC
TACGAGGCACTACGTACTCCGAGACGTGTTGGTGATGTGTGTCTTCTCGGAGAGGGACAG
  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S >
_____IGG4 CH3_____>

1570      1580      1590      1600      1610      1620
TCTGGGTAAATGAGTGCCAGGGCCGGCAAGCCCCGCTCCCCGGGCTCTCGGGGTCGCGC
AGACCCATTTACTCACGGTCCCGCCGTTCGGGGGCGAGGGGCCCCGAGAGCCCCAGCGCG
  L  G  K  *
_____>

1630      1640      1650      1660      1670      1680
GAGGATGCTTGGCAGGTACCCCGTCTACATACTTCCCAGGCACCCAGCATGGAAATAAAG
CTCCTACGAACCGTGCATGGGGCAGATGTATGAAGGGTCCGTGGGTTCGTACCTTTATTC

                                >SV40_poly_A_
                                |
1690      1700      1710|      1720      1730      1740
CACCACCACTGCCCTGGCTCGAATTCATTGATCATAATCAGCCATACCACATTTGTAGA
GTGGGTGGTGACGGGACCGAGCTTAAGTAACTAGTATTAGTCGGTATGGTGTAAACATCT

1750      1760      1770      1780      1790      1800
GGTTTACTTGTCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAA
CCAAAATGAACGAAATTTTTTGGAGGGTGTGGAGGGGACTTGGACTTTGTATTTACTT

1810      1820      1830      1840      1850      1860
TGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAG
ACGTTAACAACAACAATTGAACAATAACGTCGAATATTACCAATGTTTATTTTCGTTATC

1870      1880      1890      1900      1910      1920
CATCACAATTTACAAATAAAGCATTTTTTTCCTGCAATTCAGTTGTGGTTTGTCCAA
GTAGTGTTTAAAGTGTTTATTTTCGTAAAAAAGTGACGTAAGATCAACACCAACAGGTT

1930
ACTCATCAATGTATCTT
TGAGTAGTTACATAGAA

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FIG. 1-2

Addition of salt reduces aggregation near pl (pH 6-7).

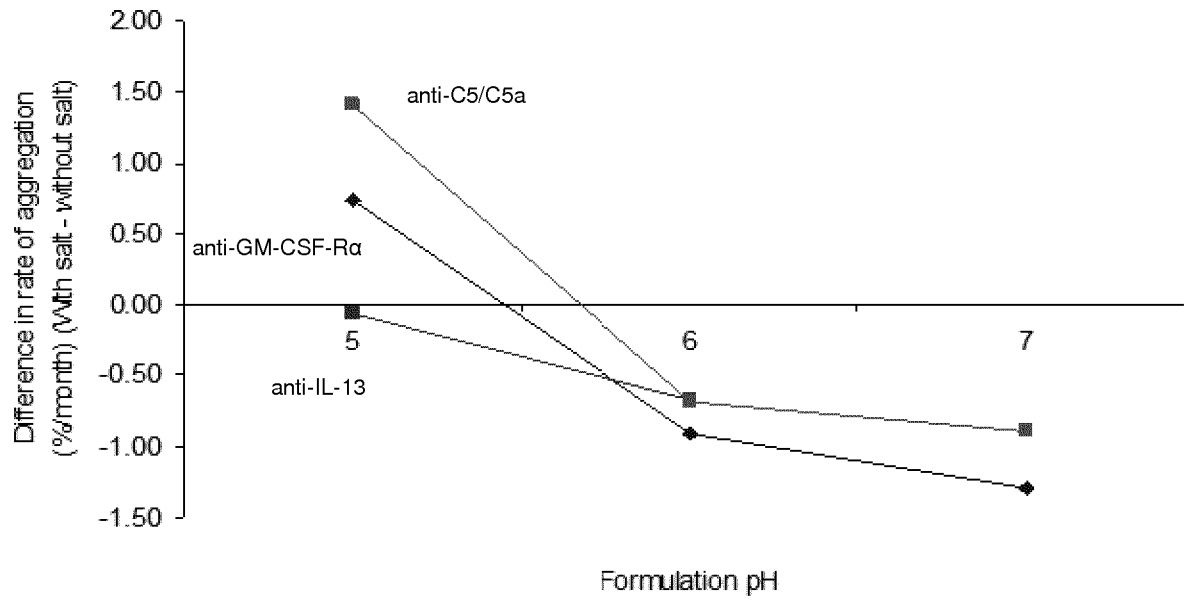


FIG. 2

IgG4 molecules effect of varying pH on  $K_D$

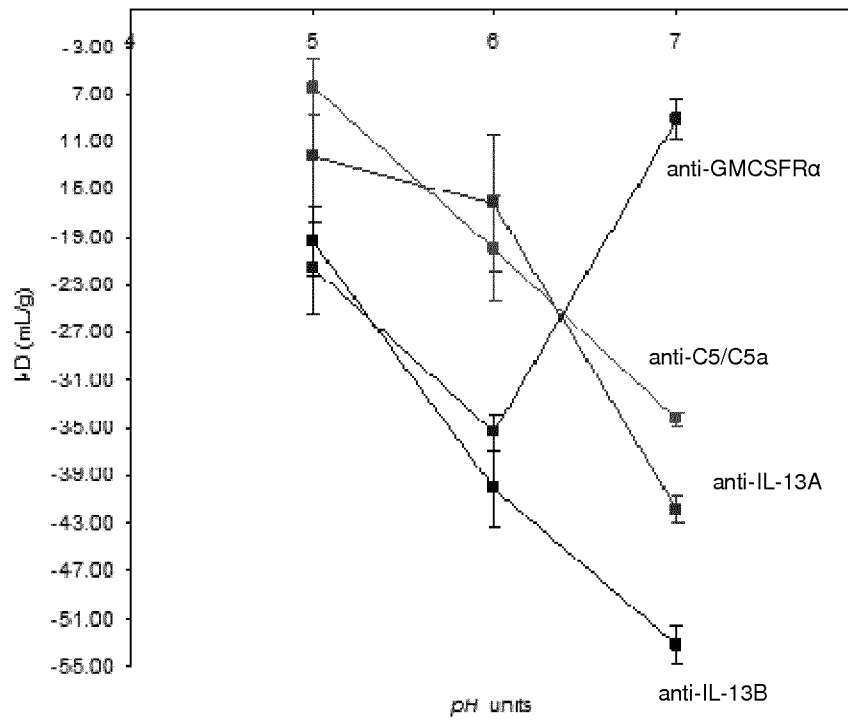


FIG. 3A

IgG4 molecules effect of varying pH on  $k_D$

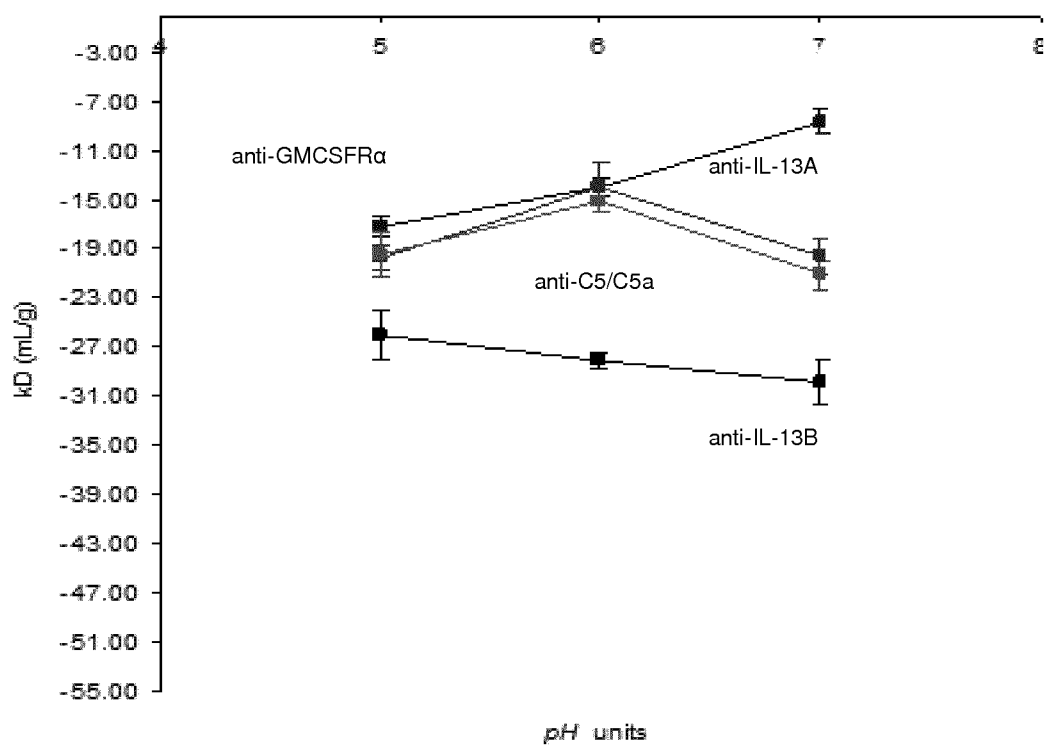


FIG. 3B

Reduction of aggregation by addition of salt is likely caused by improved colloidal stability as evidenced by increase in kD value with addition of salt near pl.

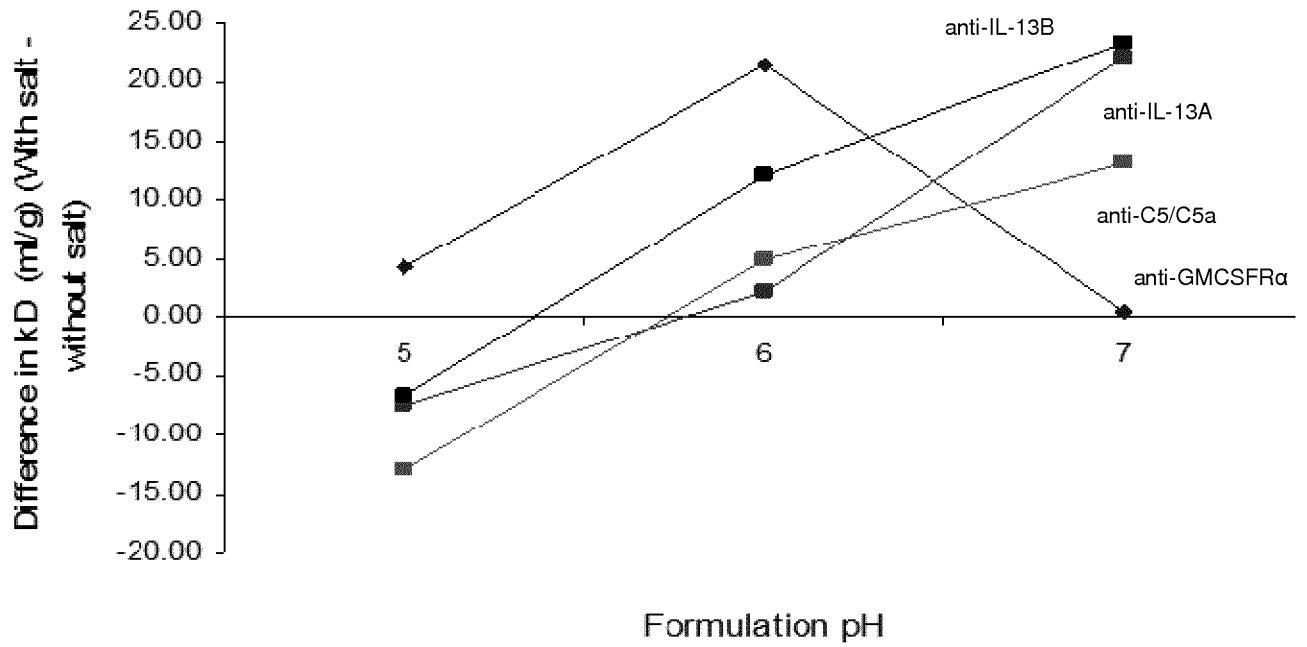


FIG. 4

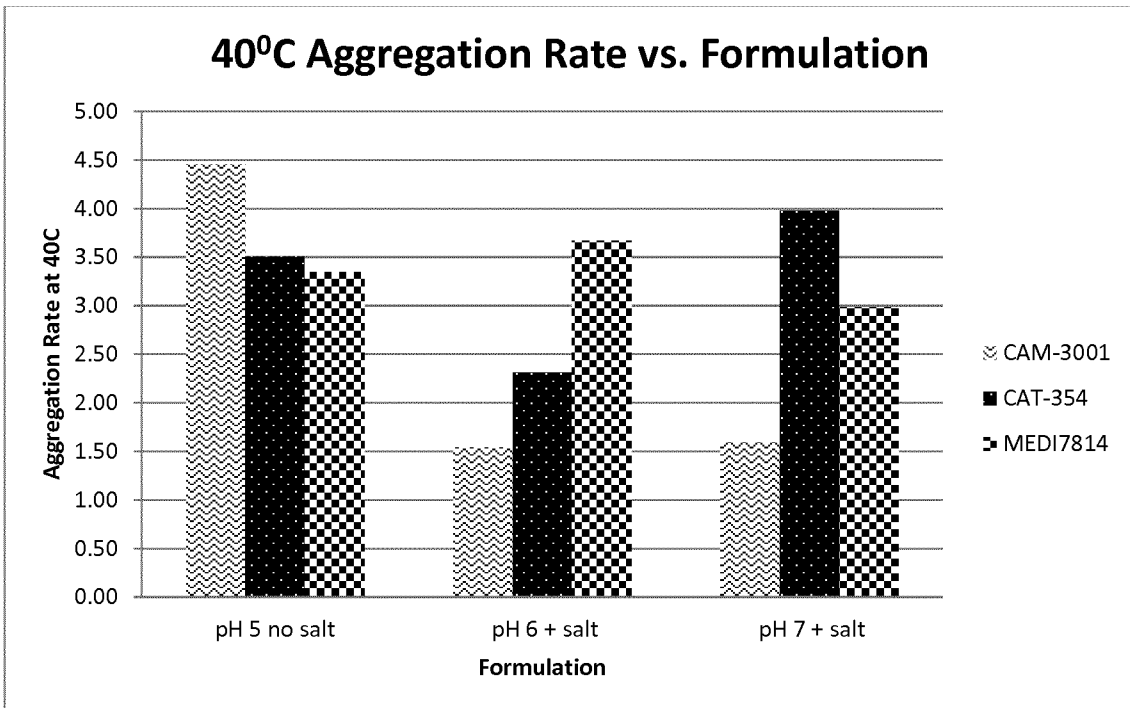


FIG. 5



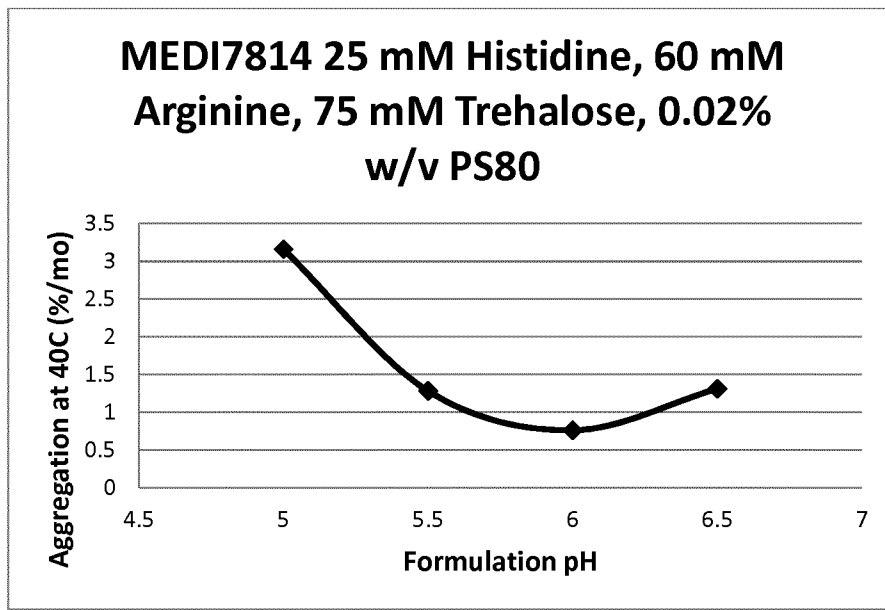


FIG. 6

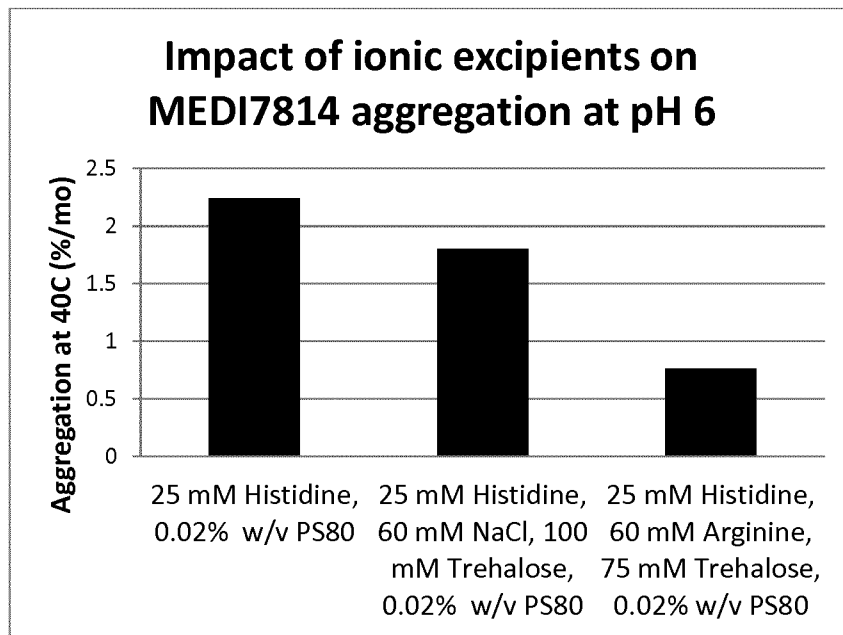


FIG. 7

**MEDI8897 heavy chain nucleotide sequence and translation**

1 Q V Q L V Q S G A E V K K P G S S V M V  
 CAA GTG CAG CTG GTG CAG TCT GGC GCC GAA GTG AAG AAA CCC GGC TCC TCC GTG ATG GTG  
 61 S C Q A S G G L L E D Y I I N W V R Q A  
 TCC TGC CAG GCT TCT GGC GGC CTG CTG GAA GAT TAC ATC ATC AAC TGG GTG CGA CAG GCC  
 121 P G Q G P E W M G G I I P V L G T V H Y  
 CCA GGC CAG GGA CCT GAA TGG ATG GGC GGA ATC ATC CCC GTG CTG GGC ACC GTG CAC TAC  
 181 G P K F Q G R V T I T A D E S T D T A Y  
GGC CCT AAG TTC CAG GGC AGA GTG ACC ATC ACC GCC GAC GAG TCT ACC GAC ACC GCC TAC  
 241 M E L S S L R S E D T A M Y Y C A T E T  
 ATG GAA CTG TCC TCC CTG CGG AGC GAG GAC ACC GCC ATG TAC TAC TGC GCC ACC GAG ACA  
 301 A L V V S E T Y L P H Y F D N W G Q G T  
GCC CTG GTG GTG TCC GAG ACA TAC CTG CCC CAC TAC TTC GAC AAC TGG GGC CAG GGA ACC  
 361 L V T V S S A S T K G P S V F P L A P S  
 CTC GTG ACC GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC  
 421 S K S T S G G T A A L G C L V K D Y F P  
 TCC AAG TCC ACC TCC GGC GGC ACC GCC GCT CTG GGC TGC CTG GTG AAG GAC TAC TTC CCT  
 481 E P V T V S W N S G A L T S G V H T F P  
 GAG CCT GTG ACC GTG TCC TGG AAC TCT GGC GCC CTG ACC TCT GGC GTG CAC ACC TTC CCT  
 541 A V L Q S S G L Y S L S S V V T V P S S  
 GCC GTG CTG CAG TCC TCC GGC CTG TAC TCC CTG TCC TCC GTG GTG ACA GTG CCT TCC TCC  
 601 S L G T Q T Y I C N V N H K P S N T K V  
 TCC CTG GGC ACC CAG ACC TAC ATC TGC AAC GTG AAC CAC AAG CCC AGC AAC ACC AAG GTG  
 661 D K R V E P K S C D K T H T C P P C P A  
 GAC AAG AGA GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA  
 721 P E L L G G P S V F L F P P K P K D T L  
 CCT GAA CTC CTG GGG GGA CCG TCA GTC TTT CTG TTC CCT CCT AAG CCT AAG GAC ACC CTG  
 781 Y I T R E P E V T C V V V D V S H E D P  
TAC ATC ACC CGG GAG CCT GAA GTG ACC TGC GTG GTG GTG GAT GTG TCC CAC GAG GAC CCT  
 841 E V K F N W Y V D G V E V H N A K T K P  
 GAG GTG AAG TTC AAT TGG TAC GTG GAC GGC GTG GAG GTG CAC AAC GCC AAG ACC AAG CCT  
 901 R E E Q Y N S T Y R V V S V L T V L H Q  
 CGG GAG GAG CAG TAC AAC TCC ACC TAC CGG GTG GTG TCT GTG CTG ACC GTG CTG CAC CAG  
 961 D W L N G K E Y K C K V S N K A L P A P  
 GAC TGG CTG AAC GGC AAA GAA TAC AAG TGC AAA GTC TCC AAC AAG GCC CTG CCT GCC CCC  
 1021 I E K T I S K A K G Q P R E P Q V Y T L  
 ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG  
 1081 P P S R E E M T K N Q V S L T C L V K G  
 CCT CCC TCC CGC GAG GAG ATG ACC AAG AAC CAG GTG TCC CTG ACC TGT CTG GTG AAG GGC  
 1141 F Y P S D I A V E W E S N G Q P E N N Y  
 TTC TAC CCT TCC GAT ATC GCC GTG GAG TGG GAG TCC AAC GGC CAG CCT GAG AAC AAC TAC  
 1201 K T T P P V L D S D G S F F L Y S K L T  
 AAG ACC ACC CCT CCT GTG CTG GAC TCC GAC GGC TCC TTC TTC CTG TAC TCC AAG CTG ACC  
 1261 V D K S R W Q Q G N V F S C S V M H E A  
 GTG GAC AAG TCC CGG TGG CAG CAG GGC AAC GTG TTC TCC TGC TCC GTG ATG CAC GAG GCT  
 1321 L H N H Y T Q K S L S L S P G K  
 CTG CAC AAC CAC TAC ACC CAG AAA AGC CTC TCC CTG TCT CCG GGT AAA

FIG. 8

**MEDI8897 light chain nucleotide sequence and translation**

D I Q M T Q S P S S L S A A V G D R V T  
 1 GAC ATC CAG ATG ACC CAG TCC CCC TCC TCT CTG TCT GCT GCC GTG GGC GAC AGA GTG ACC  
  
 I T C Q A S Q D I V N Y L N W Y Q Q K P  
 61 ATC ACC TGT CAG GCC TCC CAG GAC ATC GTG AAC TAC CTG AAC TGG TAT CAG CAG AAG CCC  
  
 G K A P K L L I Y V A S N L E T G V P S  
 121 GGC AAG GCC CCC AAG CTG CTG ATC TAC GTG GCC TCC AAC CTG GAA ACC GGC GTG CCC TCC  
  
 R F S G S G S G T D F S L T I S S L Q P  
 181 AGA TTC TCC GGC TCT GGC TCT GGC ACC GAC TTC AGC CTG ACC ATC TCC AGC CTG CAG CCT  
  
 E D V A T Y Y C Q Q Y D N L P L T F G G  
 241 GAG GAC GTG GCC ACC TAC TAC TGC CAG CAG TAC GAC AAC CTG CCC CTG ACC TTT GGC GGA  
  
 G T K V E I K R T V A A P S V F I F P P  
 301 GGC ACC AAG GTG GAG ATC AAA CGA ACT GTG GCT GCA CCA TCT GTC TTC ATC TTC CCC CCC  
  
 S D E Q L K S G T A S V V C L L N N F Y  
 361 AGC GAC GAG CAG CTG AAG AGC GGC ACC GCC TCC GTG GTG TGC CTG CTG AAC AAC TTC TAC  
  
 P R E A K V Q W K V D N A L Q S G N S Q  
 421 CCC CGC GAG GCC AAG GTG CAG TGG AAG GTG GAC AAC GCC CTG CAG TCC GGC AAC AGC CAG  
  
 E S V T E Q D S K D S T Y S L S S T L T  
 481 GAG AGC GTC ACC GAG CAG GAC AGC AAG GAC TCC ACC TAC AGC CTG AGC AGC ACC CTG ACC  
  
 L S K A D Y E K H K V Y A C E V T H Q G  
 541 CTG AGC AAG GCC GAC TAC GAG AAG CAC AAG GTG TAC GCC TGC GAG GTG ACC CAC CAG GGC  
  
 L S S P V T K S F N R G E C  
 601 CTG TCC AGC CCC GTG ACC AAG AGC TTC AAC AGG GGC GAG TGC

FIG. 9

MEDI8897 Representative Drug Product Stability

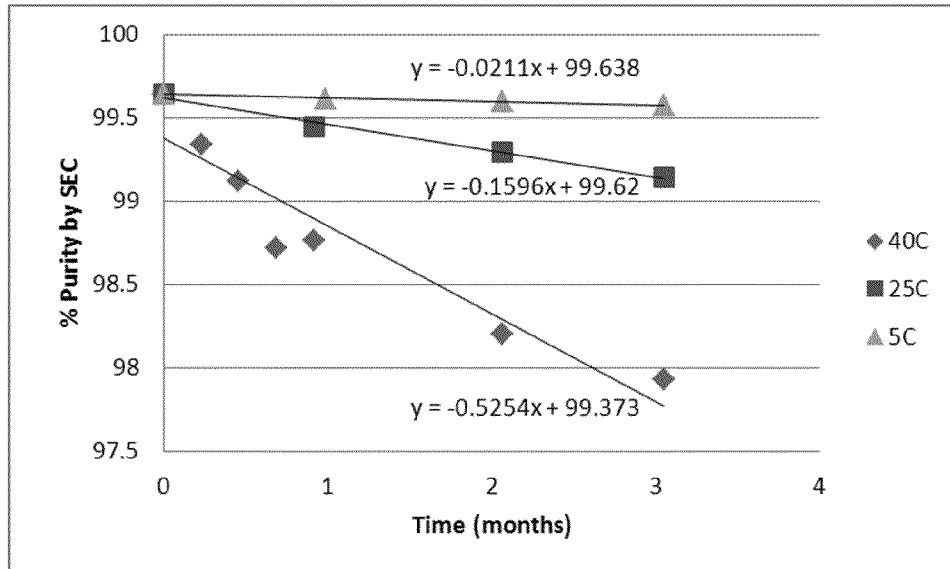


FIG. 10

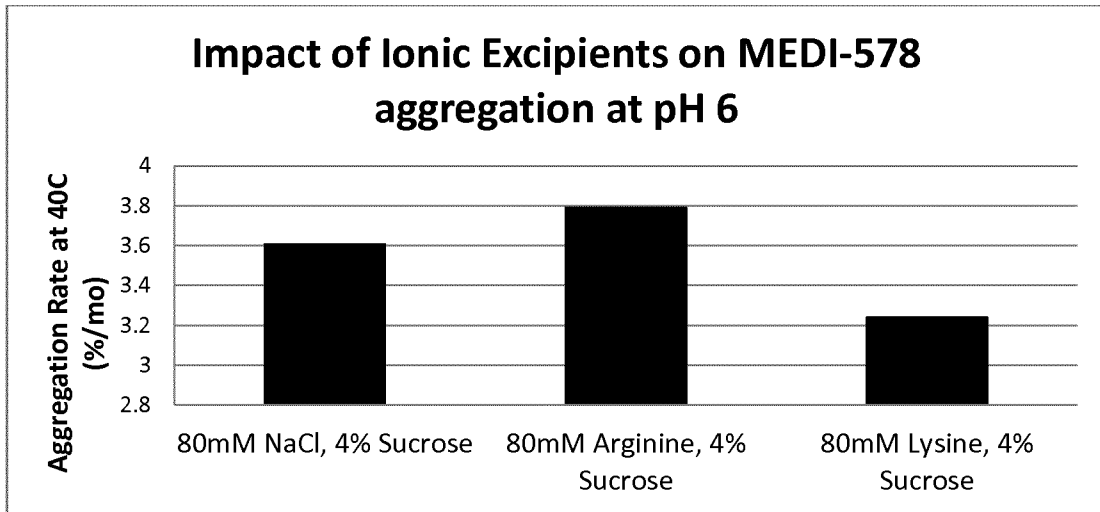
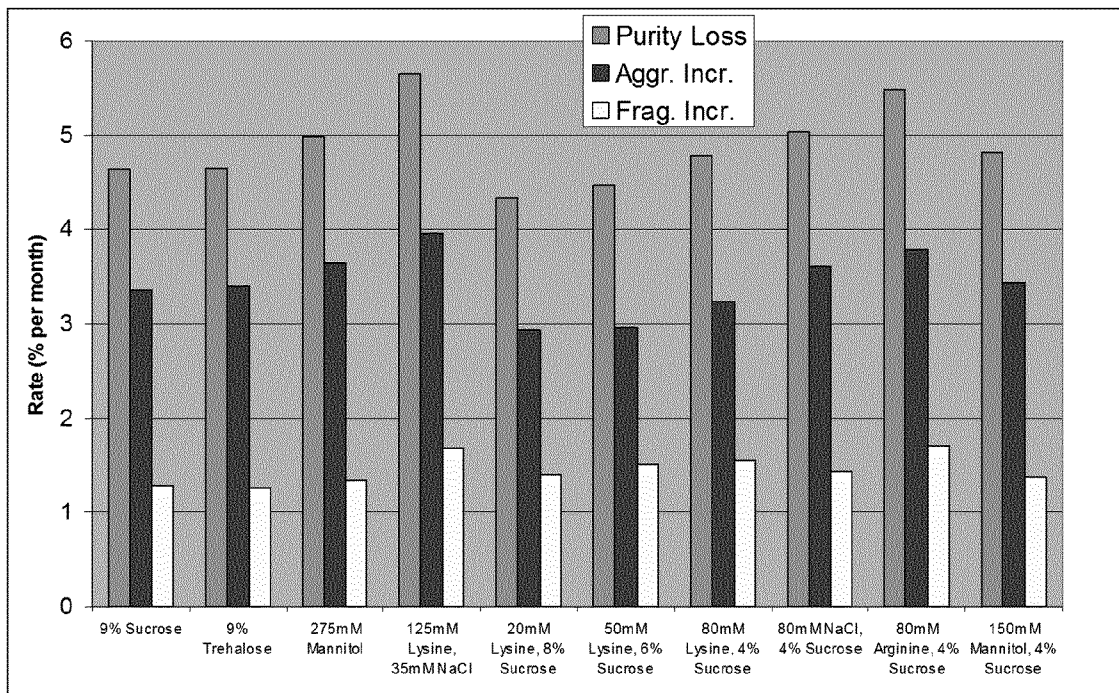


FIG. 11

Excipient Screen: Purity by SEC (4 weeks at 40°C)



■ CB historical data: (4wks – T=0 @ 40°C): 4.1%/month (mannitol), 4.5%/month (Lys/NaCl)

FIG. 12

Excipient Screen: SVP by HIAC @ 4weeks at 40°C

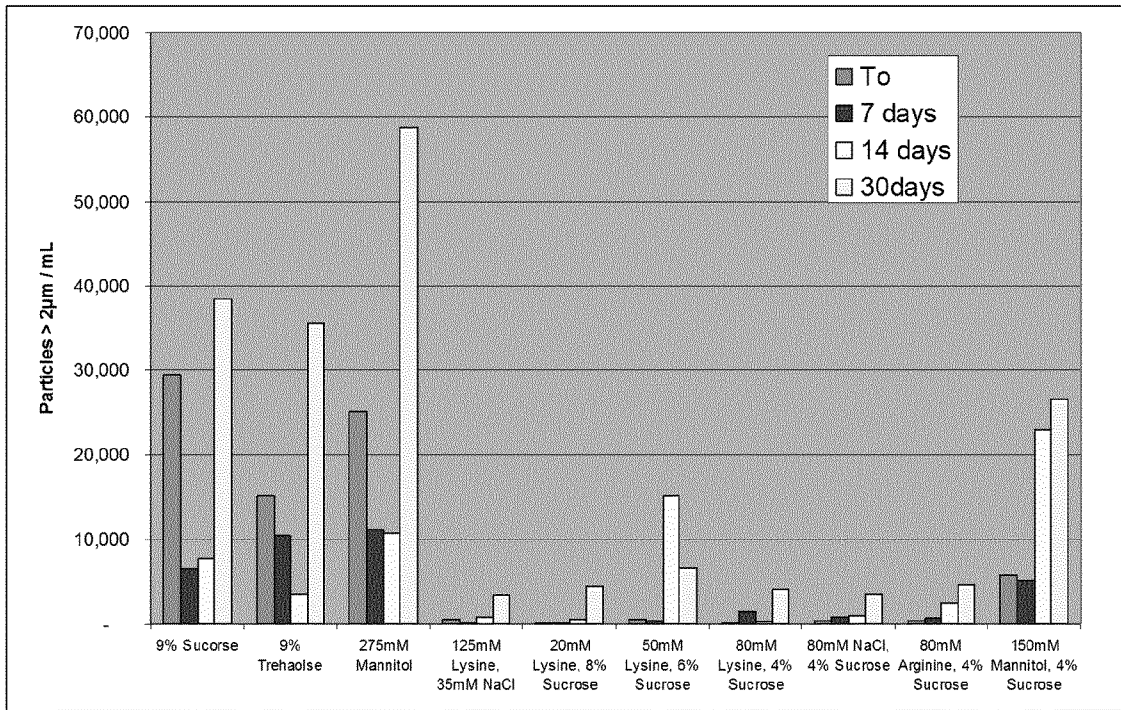


FIG. 13



INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2018/054960

A. CLASSIFICATION OF SUBJECT MATTER  
INV. A61K39/395 C07K16/24 C07K16/28 C07K16/10  
ADD.  
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED  
Minimum documentation searched (classification system followed by classification symbols)  
A61K C07K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2009/026122 A1 (AMGEN INC [US]; BONDARENKO PAVEL [US]; LI TIANSHENG [US]; SLOEY CHRIST) 26 February 2009 (2009-02-26) paragraphs [0015] - [0017], [0015], [0022], [0020], [0156] examples 1,3	35-40
X	WO 2016/059512 A1 (PFIZER [US]) 21 April 2016 (2016-04-21)	1-34, 41-46
Y	examples claims	35-40
X	WO 2013/148686 A2 (SANOFI SA [FR]; SCHNIEDERS JULIA [US]) 3 October 2013 (2013-10-03)	1-34, 41-46
Y	claims; examples	35-40
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Date of the actual completion of the international search  1 June 2018	Date of mailing of the international search report  14/06/2018
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  Covone-van Hees, M
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## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2018/054960

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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A	<p>D. ROBERTS ET AL: "Specific Ion and Buffer Effects on Protein-Protein Interactions of a Monoclonal Antibody", MOLECULAR PHARMACEUTICS, vol. 12, no. 1, 2 December 2014 (2014-12-02), pages 179-193, XP55480097, US ISSN: 1543-8384, DOI: 10.1021/mp500533c the whole document</p>	1-46
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A	<p>NEERGAARD MARTIN S ET AL: "Viscosity of high concentration protein formulations of monoclonal antibodies of the IgG1 and IgG4 subclass - Prediction of viscosity through protein-protein interaction meas", EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES, ELSEVIER, AMSTERDAM, NL, vol. 49, no. 3, 26 April 2013 (2013-04-26) , pages 400-410, XP028584050, ISSN: 0928-0987, DOI: 10.1016/J.EJPS.2013.04.019 the whole document</p>	1-46

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Information on patent family members

International application No

PCT/EP2018/054960

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		WO 2013148686 A2	03-10-2013
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