(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 October 2012 (11.10.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/138958 A1

(51) International Patent Classification:
A61K 39/00 (2006.01)

(21) International Application Number:
PCT/US2012/032462

(22) International Filing Date:
6 April 2012 (06.04.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/473,121 7 April 2011 (07.04.2011) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

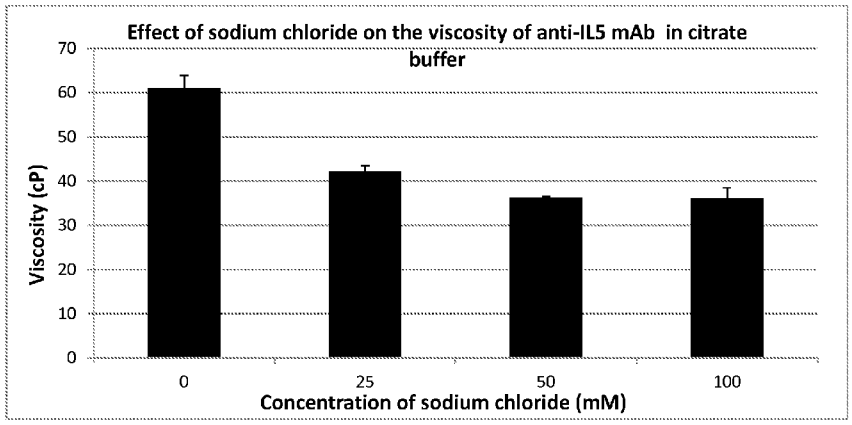
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

— *of inventorship (Rule 4.17(iv))*

[Continued on next page]

(54) Title: FORMULATIONS WITH REDUCED VISCOSITY

Figure 1



(57) Abstract: The present invention is directed to a method for reducing the viscosity of a formulation containing citrate and a therapeutic protein and formulations made using the claimed method. The present invention is also directed to a stable formulation produced by any of the methods of the present invention. The present invention is also directed to an article of manufacture comprising a container containing a formulation of the present invention.

WO 2012/138958 A1

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

FORMULATIONS WITH REDUCED VISCOSITY

Field of the Invention

The present invention relates to the field of formulations for therapeutic proteins. More specifically, the invention relates to formulations with reduced viscosity and methods of making the same.

Background of the Invention

Many drug products that comprise proteins require high therapeutic doses to achieve an efficacious patient response. In order to attain therapeutic levels in the bloodstream, therapeutic proteins, including monoclonal antibodies, are required to be administered either via intravenous or subcutaneous injection due to their size and susceptibility to proteolytic degradation. Of these two routes of administration, subcutaneous injection is more convenient for patients since drug products targeting subcutaneous routes of administration can be given at home. There are a number of monoclonal antibody drug products that have been developed either de novo or as a product line extension in pre-filled syringes for a subcutaneous route of administration. Typically, not more than 1 mL of drug product solution can be administered as a single bolus dose via a pre-filled syringe due to volume restrictions for dose administration in the subcutaneous space. However, the total volume and duration of administration is dictated by the concentration of the monoclonal antibody in the dosing solution. In order to achieve higher dose administration in smaller volumes, either for infusion or bolus administration, high concentrations of monoclonal antibodies in solution are required.

Many monoclonal antibodies in the concentration range exceeding 100mg/mL and most monoclonal antibodies at higher concentrations of 200mg/mL have relatively high viscosities leading to problems with the handling of the monoclonal antibody drug product solutions. Manufacturing processes such as tangential flow filtration for concentrating antibodies to high levels and sterile filtration are difficult and lead to yield losses for high viscosity solutions. Issues can also arise with handling and injectability of a drug product by patients or health care professionals when forces above approximately 20 Newtons must be achieved to deliver a subcutaneous dose of drug product using a prefilled syringe. It is clear that formulation approaches that give reductions in viscosity are required and the use of viscosity lowering excipients during formulation development is a viable approach.

Summary of the Invention

The present invention is directed to a method for reducing the viscosity of a formulation containing citrate and a therapeutic protein.

In one embodiment the method comprises (a) providing a formulation comprising citrate; and (b) adding sodium chloride to the formulation to a concentration of about 20 mM to about 150 mM, wherein the viscosity of the formulation with the sodium chloride is reduced compared to the viscosity of the same formulation without sodium chloride.

In another embodiment the method comprises (a) providing a formulation comprising citrate; and (b) adding glycine and/or arginine to the formulation to a concentration of about 0.4% w/v to about 1.1% w/v, wherein the viscosity of the formulation with the glycine and/or arginine is reduced compared to the viscosity of the same formulation without glycine and/or arginine.

In another embodiment the method comprises (a) providing a formulation comprising citrate; and (b) adding phenylalanine to the formulation to a concentration of about 0.4% w/v to about 1.1% w/v, wherein the viscosity of the formulation with the phenylalanine is reduced compared to the viscosity of the same formulation without phenylalanine.

In another embodiment the method comprises (a) providing a formulation comprising citrate; and (b) adding tyrosine to the formulation to a concentration of about 0.001% w/v to about 0.005% w/v, wherein the viscosity of the formulation with the tyrosine is reduced compared to the viscosity of the same formulation without tyrosine.

In another embodiment the method comprises (a) providing a formulation comprising citrate; and (b) adding proline to the formulation to a concentration of about 4.0% w/v , wherein the viscosity of the formulation with the proline is reduced compared to the viscosity of the same formulation without proline.

The present invention is also directed to a stable formulation produced by any of the methods of the present invention.

The present invention is also directed to an article of manufacture comprising a container containing a formulation of the present invention.

Brief Description of the Drawings

Figure 1. Sodium chloride: At all the concentrations tested, sodium chloride reduced the viscosity of anti-IL5 mAb in citrate buffer.

Figure 2. Citrate buffer: All concentrations of linear chain amino acids reduced the viscosity of the samples except 0.04% methionine.

Figure 3. Citrate: Phenylalanine, tyrosine and proline reduced the viscosity of anti-IL5 mAb formulations but tryptophan did not change the viscosity of anti-IL5 mAb formulations.

Detailed Description of the Invention

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

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

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

The present invention is directed to a method for reducing the viscosity of a formulation containing citrate and a therapeutic protein.

In exemplary embodiments of the present invention, the liquid polypeptide compositions that are produced exhibit desirable characteristics, such as desirable viscosity and surface tension characteristics.

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

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

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

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

In certain embodiments, the formulation with reduced viscosity has a viscosity less than about 50 cP, less than about 45 cP, less than about 40 cP, less than about 35 cP, less than about 30 cP, less than about 25 cP, less than about 20 cP, or less than about 15 cP.

"Polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. A polypeptide can be of natural (tissue-derived) origins, recombinant or natural expression from prokaryotic or eukaryotic cellular preparations, or produced chemically via synthetic methods. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally

occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid. Non-natural residues are well described in the scientific and patent literature; a few exemplary non-natural compositions useful as mimetics of natural amino acid residues and guidelines are described below. Mimetics of aromatic amino acids can be generated by replacing by, e.g., D- or L-naphylalanine; D- or L-phenylglycine; D- or L-2 thieneylalanine; D- or L-1, -2,3-, or 4-pyreneylalanine; D- or L-3 thieneylalanine; D- or L-(2-pyridinyl)-alanine; D- or L-(3-pyridinyl)-alanine; D- or L-(2-pyrazinyl)-alanine; D- or L-(4-isopropyl)-phenylglycine: D-(trifluoromethyl)-phenylglycine; D-(trifluoromethyl)-phenylalanine: D-p-fluoro-phenylalanine; D- or L-p-biphenylphenylalanine; K- or L-p-methoxy-biphenylphenylalanine: D- or L-2-indole(alkyl)alanines; and, D- or L-alkylainines, where alkyl can be substituted or unsubstituted methyl, ethyl, propyl, hexyl, butyl, pentyl, isopropyl, iso-butyl, sec-isotyl, iso-pentyl, or non-acidic amino acids. Aromatic rings of a non-natural amino acid include, e.g., thiazolyl, thiophenyl, pyrazolyl, benzimidazolyl, naphthyl, furanyl, pyrrolyl, and pyridyl aromatic rings.

"Peptide" as used herein includes peptides which are conservative variations of those peptides specifically exemplified herein. "Conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include, but are not limited to, the substitution of one hydrophobic residue such as isoleucine, valine, leucine, alanine, cysteine, glycine, phenylalanine, proline, tryptophan, tyrosine, norleucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. Neutral hydrophilic amino acids which can be substituted for one another include asparagine, glutamine, serine and threonine. "Conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide. Such conservative substitutions are within the definition of the classes of the peptides of the invention. "Cationic" as used herein refers to any peptide that possesses a net positive charge at pH 7.4. The biological activity of the peptides can be determined by standard methods known to those of skill in the art and described herein.

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

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

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

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

Table 1. Amino acid abbreviations.

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

In another embodiment the polypeptide is an antigen binding polypeptide. In one embodiment the antigen binding polypeptide is selected from the group consisting of a soluble receptor, antibody, antibody fragment, immunoglobulin single variable domain, Fab, F(ab')2, Fv, disulphide linked Fv, scFv, closed conformation multispecific antibody, disulphide-linked scFv, or diabody.

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

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

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

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

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

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

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate). The structure and protein folding of the antibody may mean that other residues are considered part of the antigen binding region and would be understood to be so by a skilled person. See for example Chothia et al., (1989) Conformations of immunoglobulin hypervariable regions; Nature 342, p 877-883.

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

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

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

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

The terms "mAbdAb" and dAbmAb" are used herein to refer to antigen-binding proteins of the present invention. The two terms can be used interchangeably, and are intended to have the same meaning as used herein.

In one embodiment the method comprises (a) providing a formulation comprising citrate; and (b) adding sodium chloride to the formulation to a concentration of about 20 mM to about

150 mM, wherein the viscosity of the formulation with the sodium chloride is reduced compared to the viscosity of the same formulation without sodium chloride. In certain embodiments, the sodium chloride is added to a concentration selected from the group consisting of about 25 mM, about 50 mM, and about 100mM. In certain embodiments, the viscosity of the formulation with sodium chloride is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, or at least about 40% compared to the viscosity of the formulation in the absence of sodium chloride. In certain embodiments, the viscosity of the formulation with sodium chloride is less than about 50 cP, less than about 45 cP, less than about 40 cP, or less than about 35 cP.

In one embodiment the method comprises (a) providing a formulation comprising citrate; and (b) adding an amino acid or multiple amino acids to the formulation, wherein the viscosity of the formulation with the amino acid(s) is reduced compared to the viscosity of the same formulation without the same amino acids(s). In certain embodiments the amino acid(s) is a linear amino acid. In other embodiments the amino acid comprises a cyclic portion. In one embodiment the amino acid(s) is tyrosine, glycine, phenylalanine, methionine, alanine, serine, isoleucine, leucine, threonine, valine, proline, lysine, histidine, glutamine, glutamic acid, arginine, aspartic acid, asparagine, cysteine.

In one embodiment the method comprises (a) providing a formulation comprising citrate; and (b) adding glycine and/or arginine to the formulation to a concentration of about 0.4% w/v to about 1.1% w/v, wherein the viscosity of the formulation with the glycine and/or arginine is reduced compared to the viscosity of the same formulation without glycine and/or arginine. In certain embodiments, the glycine and/or arginine is added to a concentration selected from the group consisting of about 0.5% w/v and about 1% w/v. In certain embodiments, the viscosity of the formulation with glycine and/or arginine is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, or at least about 50% compared to the viscosity of the formulation in the absence of glycine and/or arginine. In certain embodiments, the viscosity of the formulation with glycine and/or arginine is less than about 50 cP, less than about 45 cP, less than about 40 cP, less than about 35 cP, less than about 30 cP, or less than about 25 cP.

In another embodiment the method comprises (a) providing a formulation comprising citrate; and (b) adding phenylalanine to the formulation to a concentration of about 0.4% w/v to about 1.1% w/v, wherein the viscosity of the formulation with the phenylalanine is reduced

compared to the viscosity of the same formulation without phenylalanine. In certain embodiments, the phenylalanine is added to a concentration of about 0.8% w/v. In certain embodiments, the viscosity of the formulation with phenylalanine is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, or at least about 50% compared to the viscosity of the formulation in the absence of phenylalanine. In certain embodiments, the viscosity of the formulation with phenylalanine is less than about 50 cP, less than about 45 cP, less than about 40 cP, less than about 35 cP, less than about 30 cP, or less than about 25 cP.

In another embodiment the method comprises (a) providing a formulation comprising citrate; and (b) adding tyrosine to the formulation to a concentration of about 0.001% w/v to about 0.005% w/v, wherein the viscosity of the formulation with the tyrosine is reduced compared to the viscosity of the same formulation without tyrosine. In certain embodiments, the tyrosine is added to a concentration of about 0.004% w/v. In certain embodiments, the viscosity of the formulation with tyrosine is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, or at least about 50% compared to the viscosity of the formulation in the absence of tyrosine. In certain embodiments, the viscosity of the formulation with tyrosine is less than about 50 cP, less than about 45 cP, less than about 40 cP, less than about 35 cP, less than about 30 cP, or less than about 25 cP.

In another embodiment the method comprises (a) providing a formulation comprising citrate; and (b) adding methionine to the formulation to a concentration of about 0.01% w/v. In certain embodiments, the viscosity of the formulation with methionine is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, compared to the viscosity of the formulation in the absence of methionine. In certain embodiments, the viscosity of the formulation with methionine is less than about 50 cP, less than about 45 cP, less than about 40 cP, or less than about 35 cP.

In another embodiment the method comprises (a) providing a formulation comprising citrate; and (b) adding proline to the formulation to a concentration of about 4.0%w/v, wherein the viscosity of the formulation with the proline is reduced compared to the viscosity of the same formulation without proline. In certain embodiments, the viscosity of the formulation with proline is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least

about 60%, at least about 70%, or at least about 75% compared to the viscosity of the formulation in the absence of proline. In certain embodiments, the viscosity of the formulation with proline is less than about 50 cP, less than about 45 cP, less than about 40 cP, less than about 35 cP, less than about 30 cP, less than about 25 cP, less than about 20 cP, or less than about 15 cP.

In another embodiment the method further comprises determining the stability of the protein formulation.

In another embodiment the formulation further comprises additional excipients. "Excipients" includes, but is not limited to, stabilizers, for example, human serum albumin (hsa), bovine serum albumin (bsa), α-casein, globulins, α-lactalbumin, LDH, lysozyme, myoglobin, ovalbumin, RNase A; buffering agents, for example, citric acid, HEPES, histidine, potassium acetate, postassium citrate, potassium phosphate (KH_2PO_4), sodium acetate, sodium bicarbonate, sodium citrate, sodium phosphate (NAH_2PO_4), Tris base, and Tris–HCl; amino acids/metabolites, for example, glycine, alanine (α-alanine, β-alanine), arginine, betaine, leucine, lysine, glutamic acid, aspartic acid, histidine, proline, 4-hydroxyproline, sarcosine, γ-aminobutyric acid (GABA), opines (alanopine, octopine, strombine), and trimethylamine N-oxide (TMAO); surfactants, for example, polysorbate 20 and 80, and poloxamer 407: lipid molecules, for example, phosphatidyl choline, ethanolamine, and acethyltryptophanate: polymers, for example, polyethylene glycol (PEG), and polyvinylpyrrolidone (PVP) 10, 24, 40; low molecular weight excipients, for example, arabinose, cellobiose, ethylene glycol, fructose, fucose, galactose, glycerin/glycerol, glucose, inositol, lactose, mannitol, maltose, maltotriose, mannose, melibiose, 2-methyl-2,4-pentanediol, octulose, propylene glycol, raffinose , ribose, sorbitol, sucrose, trehalose, xylitol, and xylose; and high molecular weight excipients, for example, cellulose, β-cyclodextrin, dextran (10 kd), dextran (40 kd), dextran (70 kd), ficoll, gelatin, hydroxypropylmethyl-cellulose, hydroxyethyl starch, maltodextrin, methocel, peg (6 kd), polydextrose, polyvinylpyrrolidone (PVP) k15 (10 kd), PVP (40 kd), PVP k30 (40 kd), PVP k90 (1000 kd), sephadex G 200, and starch; antioxidants, for example, ascorbic acid, cysteine HCl, thioglycerol, thioglycolic acid, thiosorbitol, and glutathione; reducing agents, for example, cysteine HCl, dithiothreotol, and other thiol or thiophenes; chelating agents, for example, EDTA, EGTA, glutamic acid, and aspartic acid; inorganic salts/metals, for example, Ca^{2+}, Ni^{2+}, Mg^{2+}, Mn^{2+}, Na_2SO_4, $(NH_4)_2SO_4$, Na_2HPO_4/NaH_2PO_4, K_2HPO_4/KH_2PO_4, $MgSO_4$, and NaF; organic salts, for example, Na acetate, Na polyethylene, Na caprylate (Na octanoate), proprionate, lactate, succinate, and citrate; organic solvents, for example, acetonitrile, dimethylsulfoxide (dmso), and ethanol.

In one embodiment the formulation further comprises sucrose. In one embodiment the formulation comprises sucrose at a concentration of about 150 to about 300 mM. In one embodiment the formulation comprises sucrose at a concentration of about 200 to about 250 mM. In one embodiment the formulation comprises sucrose at a concentration of about 234 mM.

In one embodiment the formulation is formulated to a pH of about 5.0 to about 8.0. In one embodiment the formulation is formulated to a pH of about 6.0. In one embodiment the formulation comprises about 10 mM to about 50 mM citrate. In one embodiment the formulation comprises about 20 mM citrate.

In another embodiment the formulation further comprises polysorbate-80. In one embodiment the formulation further comprises polysorbate-80 at a concentration of up to 0.05% w/v.

In another embodiment the therapeutic protein is an antigen binding polypeptide. In one embodiment the antigen binding polypeptide is an antibody. In one embodiment the antigen binding polypeptide is an immunoglobulin single variable domain. In one embodiment the antigen binding polypeptide binds to interleukin 5 (IL5). In one embodiment the antigen binding polypeptide is an anti-IL5 antibody. In one embodiment the anti-IL5 antibody comprises a heavy chain comprising SEQ ID NO:1 and a light chain comprising SEQ ID NO:2.

In another embodiment the therapeutic protein is present at a concentration of at least about 150 mg/ml, at least about 175 mg/ml, at least about 200 mg/ml, at least about 225 mg/ml, at least about 250 mg/ml, at least about 275 mg/ml, or at least about 300 mg/ml. In another embodiment the therapeutic protein is present at a concentration of at least about 150 mg/ml to about 300 mg/ml. In one embodiment the therapeutic protein is present at a concentration of about 200 mg/ml.

In one embodiment the formulation is lyophilized or spray dried, and then reconstituted before the viscosity is determined. In certain embodiments the formulation with reduced viscosity is lyophilized or spray dried and then later reconstituted with a dispersing agent. In one embodiment the dispersing agent is sterile water or “water for injection” (WFI). The liquid polypeptide can be further diluted with isotonic saline or other excipients to produce a desirable concentration prior to administration. In one embodiment the formulation is a reconstituted formulation. In another embodiment the formulation is a liquid pharmaceutical formulation.

The agents used to reduce viscosity can be added at any stage of the formulation process. For example, before, after, or concurrently with the citrate, the therapeutic protein, or with any excipients.

The formulations of the present invention may be administered by any suitable route of administration, including systemic administration. Systemic administration includes oral administration, parenteral administration, transdermal administration, rectal administration, and administration by inhalation. Parenteral administration refers to routes of administration other than enteral, transdermal, or by inhalation, and is typically by injection or infusion. Parenteral administration includes intravenous, intramuscular, and subcutaneous injection or infusion. Inhalation refers to administration into the patient's lungs whether inhaled through the mouth or through the nasal passages.

The present invention is also directed to a stable formulation produced by any of the methods of the present invention.

In one embodiment the formulation comprises citrate, the therapeutic protein, and sodium chloride. In one embodiment the concentration of sodium chloride is about 20 mM to about 150 mM, wherein the viscosity of the formulation with the sodium chloride is reduced compared to the viscosity of the same formulation without sodium chloride. In certain embodiments, the concentration of sodium chloride is about 25 mM, about 50 mM, or about 100mM. In certain embodiments, the viscosity of the formulation with sodium chloride is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, or at least about 40% compared to the viscosity of the formulation in the absence of sodium chloride. In certain embodiments, the viscosity of the formulation with sodium chloride is less than about 50 cP, less than about 45 cP, less than about 40 cP, or less than about 35 cP.

In one embodiment the formulation comprises citrate, the therapeutic protein, and an amino acid or multiple amino acids, wherein the viscosity of the formulation with the amino acid(s) is reduced compared to the viscosity of the same formulation without the same amino acids(s). In certain embodiments the amino acid(s) is a linear amino acid. In other embodiments the amino acid comprises a cyclic portion. In one embodiment the amino acid(s) is tyrosine, glycine, phenylalanine, methionine, alanine, serine, isoleucine, leucine, threonine, valine, proline, lysine, histidine, glutamine, glutamic acid, arginine, aspartic acid, asparagine, cysteine.

In one embodiment the formulation comprises citrate, the therapeutic protein, and glycine and/or arginine. In one embodiment the concentration of glycine and/or arginine is about 0.4% w/v to about 1.1% w/v, wherein the viscosity of the formulation with the glycine and/or arginine is reduced compared to the viscosity of the same formulation without glycine and/or arginine. In one embodiment the concentration of glycine and/or arginine is about 0.5% w/v or about 1% w/v. In certain embodiments, the viscosity of the formulation with glycine and/or arginine is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, or at least about 50% compared to the viscosity of the formulation in the absence of glycine and/or arginine. In certain embodiments, the viscosity of the formulation with glycine and/or arginine is less than about 50 cP, less than about 45 cP, less than about 40 cP, less than about 35 cP, less than about 30 cP, or less than about 25 cP.

In one embodiment the formulation comprises citrate, the therapeutic protein, and phenylalanine. In one embodiment the concentration of phenylalanine is about 0.4% w/v to about 1.1% w/v, wherein the viscosity of the formulation with the phenylalanine is reduced compared to the viscosity of the same formulation without phenylalanine. In certain embodiments, the concentration of phenylalanine is about 0.8% w/v. In certain embodiments, the viscosity of the formulation with phenylalanine is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, or at least about 50% compared to the viscosity of the formulation in the absence of phenylalanine. In certain embodiments, the viscosity of the formulation with phenylalanine is less than about 50 cP, less than about 45 cP, less than about 40 cP, less than about 35 cP, less than about 30 cP, or less than about 25 cP.

In one embodiment the formulation comprises citrate, the therapeutic protein, and tyrosine. In one embodiment the concentration of tyrosine is about 0.001% w/v to about 0.005% w/v, wherein the viscosity of the formulation with the tyrosine is reduced compared to the viscosity of the same formulation without tyrosine. In certain embodiments, the concentration of tyrosine is about 0.004% w/v. In certain embodiments, the viscosity of the formulation with tyrosine is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, or at least about 50% compared to the viscosity of the formulation in the absence of tyrosine. In certain embodiments, the viscosity of the formulation with tyrosine is less than about 50 cP, less than about 45 cP, less than about 40 cP, less than about 35 cP, less than about 30 cP, or less than about 25 cP.

In one embodiment the formulation comprises citrate, the therapeutic protein, and methionine. In one embodiment the concentration of methionine is about 0.01% w/v, wherein the viscosity of the formulation with the methionine is reduced compared to the viscosity of the same formulation without tyrosine. In certain embodiments, the viscosity of the formulation with methionine is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, compared to the viscosity of the formulation in the absence of methionine. In certain embodiments, the viscosity of the formulation with methionine is less than about 50 cP, less than about 45 cP, less than about 40 cP, or less than about 35 cP.

In one embodiment the formulation comprises citrate, the therapeutic protein, and proline. In one embodiment the concentration of proline is about 4.0% w/v, wherein the viscosity of the formulation with the proline is reduced compared to the viscosity of the same formulation without proline. In certain embodiments, the viscosity of the formulation with proline is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, or at least about 75% compared to the viscosity of the formulation in the absence of proline. In certain embodiments, the viscosity of the formulation with proline is less than about 50 cP, less than about 45 cP, less than about 40 cP, less than about 35 cP, less than about 30 cP, less than about 25 cP, less than about 20 cP, or less than about 15 cP.

In one embodiment the formulation further comprises sucrose. In one embodiment the formulation comprises sucrose at a concentration of about 150 to about 300 mM. In one embodiment the formulation comprises sucrose at a concentration of about 200 to about 250 mM. In one embodiment the formulation comprises sucrose at a concentration of about 234 mM.

In one embodiment the formulation is formulated to a pH of about 5.0 to about 8.0. In one embodiment the formulation is formulated to a pH of about 6.0. In one embodiment the formulation comprises about 10 mM to about 50 mM citrate. In one embodiment the formulation comprises about 20 mM citrate.

In another embodiment the formulation further comprises polysorbate-80. In another embodiment the formulation further comprises polysorbate-80. In one embodiment the formulation further comprises polysorbate-80 at a concentration of up to 0.05% w/v.

The present invention is also directed to an article of manufacture comprising a container containing a formulation of the present invention. In one embodiment the article of manufacture further comprises directions for administration of the formulation.

Examples

Glycine, tyrosine, tryptophan, phenylalanine, and proline were acquired from Sigma-Aldrich. Arginine was acquired from MP-Biomedicals and methionine was acquired from JT Baker. All the amino acids were laboratory grade. Anti-IL5 mAb stock (220 mg/mL) solutions were prepared in-house and were formulated with 234mM sucrose in citrate buffer (pH 6.0).

The concentration of the anti-IL5 mAb solution was adjusted to 200 mg/mL for viscosity measurements as described below. For sodium chloride, glycine, arginine, methionine and tyrosine, stock solutions were prepared in citrate buffers (Tables 2a and b) and spiked into the 220mg/mL anti-IL5 mAb stock solution of the respective buffer (Tables 3a and b).

For tryptophan, phenylalanine, and proline the amino acids were dissolved directly into the anti-IL5 mAb solution so as to attain the targeted amino acid concentration in Table 3b. The concentrations could not be attained by making a stock solution due to their low water solubility.

Table 2a: Concentration of stock solution of salt.

Name of salt	Concentration of stock solution in citrate buffers (M)
Sodium chloride	2.0

Table 2b: Concentrations of stock solutions of amino acids.

Name of amino acid	Concentration of stock solution in citrate buffers (% w/v)
Glycine	10.90
Arginine	10.90
Methionine	0.80
Tyrosine	0.04

Table 3a: Dilution scheme of salt to attain 200 mg/mL anti-IL5 mAb with salt.

	Salt concentration in the final 200mg/mL anti-IL5 mAb solution (mM)	Volume of 220mg/mL anti-IL5 mAb stock (µL)	Volume of salt stock (µL)	Volume of citrate buffer (µL)
Sodium chloride	100	4545	250	205
Sodium chloride	50	4545	125	330
Sodium chloride	25	4545	62.5	392.5

Table 3b: Dilution scheme of amino acids to attain 200 mg/mL anti-IL5 mAb with the amino acid concentrations.

	Amino acid concentration in the final 200mg/mL anti-IL5 mAb solution (% w/v)	Volume of 220mg/mL anti-IL5 mAb stock (µL)	Volume of amino acid stock (µL)	Volume of citrate buffer (µL)	Weight of amino acid (g)
Glycine	0.5	1818	91	91	NA
Glycine	1.0	1818	182	0	NA
Arginine	0.5	1818	91	91	NA
Arginine	1.0	1818	182	0	NA
Methionine	0.01	1818	25	157	NA
Methionine	0.04	1818	100	82	NA
Tryptophan	0.2	9090	NA	910	0.05
Phenylalanine	0.83	5454	NA	546	0.02
Tyrosine	0.004	1818	182	0	NA
Proline	4.0	9090	NA	900	0.4

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Following sample dilution, the viscosity of the samples was measured with a Brookfield LVDVUUltra III C/P rheometer at 25 °C. The spindle used was CP-40 and 500 µL of sample was loaded for each measurement. Mean viscosity values were calculated from viscosity values obtained that were unchanged with increases in % torque.

What is claimed is:

1. A method for reducing the viscosity of a formulation containing citrate and a therapeutic protein, the method comprising; (a) providing a formulation comprising citrate; and (b) adding sodium chloride to the formulation to a concentration of about 20 mM to about 150 mM, wherein the viscosity of the formulation with the sodium chloride is reduced compared to the viscosity of the same formulation without sodium chloride.

2. The method of claim 1 wherein the sodium chloride is added to a concentration selected from the group consisting of about 25 mM, about 50 mM, and about 100mM.

3. The method of any preceding claim, wherein the viscosity of the formulation with sodium chloride is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, or at least about 40% compared to the viscosity of the formulation in the absence of sodium chloride.

4. The method of any preceding claim, wherein the viscosity of the formulation with sodium chloride is less than about 50 cP, less than about 45 cP, less than about 40 cP, or less than about 35 cP.

5. A method for reducing the viscosity of a formulation containing citrate and a therapeutic protein, the method comprising; (a) providing a formulation comprising citrate; and (b) adding glycine and/or arginine to the formulation to a concentration of about 0.4% w/v to about 1.1% w/v, wherein the viscosity of the formulation with the glycine and/or arginine is reduced compared to the viscosity of the same formulation without glycine and/or arginine.

6. The method of claim 5 wherein the glycine and/or arginine is added to a concentration selected from the group consisting of about 0.5% w/v and about 1% w/v.

7. The method of claim 5 or 6, wherein the viscosity of the formulation with glycine and/or arginine is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, or at least about 50% compared to the viscosity of the formulation in the absence of glycine and/or arginine.

8. The method of claim 5, 6, or 7, wherein the viscosity of the formulation with glycine and/or arginine is less than about 50 cP, less than about 45 cP, less than about 40 cP, less than about 35 cP, less than about 30 cP, or less than about 25 cP.

9. A method for reducing the viscosity of a formulation containing citrate and a therapeutic protein, the method comprising; (a) providing a formulation comprising citrate; and (b) adding phenylalanine to the formulation to a concentration of about 0.4% w/v to about 1.1% w/v, wherein the viscosity of the formulation with the phenylalanine is reduced compared to the viscosity of the same formulation without phenylalanine.

10. The method of claim 9 wherein the phenylalanine is added to a concentration of about 0.8% w/v.

11. The method of claim 9 or 10, wherein the viscosity of the formulation with phenylalanine is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, or at least about 50% compared to the viscosity of the formulation in the absence of phenylalanine.

12. The method of claim 9, 10, or 11, wherein the viscosity of the formulation with phenylalanine is less than about 50 cP, less than about 45 cP, less than about 40 cP, less than about 35 cP, less than about 30 cP, or less than about 25 cP.

13. A method for reducing the viscosity of a formulation containing citrate and a therapeutic protein, the method comprising; (a) providing a formulation comprising citrate; and (b) adding tyrosine to the formulation to a concentration of about 0.001% w/v to about 0.005% w/v, wherein the viscosity of the formulation with the tyrosine is reduced compared to the viscosity of the same formulation without tyrosine.

14. The method of claim 13 wherein the tyrosine is added to a concentration of about 0.004% w/v.

15. The method of claim 13 or 14, wherein the viscosity of the formulation with tyrosine is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, or at least about 50% compared to the viscosity of the formulation in the absence of tyrosine.

16. The method of claim 13, 14, or 15, wherein the viscosity of the formulation with tyrosine is less than about 50 cP, less than about 45 cP, less than about 40 cP, less than about 35 cP, less than about 30 cP, or less than about 25 cP.

17. A method for reducing the viscosity of a formulation containing citrate and a therapeutic protein, the method comprising; (a) providing a formulation comprising citrate; and (b) adding

proline to the formulation to a concentration of about 4.0% w/v, wherein the viscosity of the formulation with the proline is reduced compared to the viscosity of the same formulation without proline.

18. The method of claim 17, wherein the viscosity of the formulation with proline is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, or at least about 75% compared to the viscosity of the formulation in the absence of proline.

19. The method of claim 17 or 18, wherein the viscosity of the formulation with tyrosine is less than about 50 cP, less than about 45 cP, less than about 40 cP, less than about 35 cP, less than about 30 cP, less than about 25 cP, less than about 20 cP, or less than about 15 cP.

20. The method of any preceding claim, wherein the method further comprises determining the stability of the protein formulation.

21. The method of any preceding claim, wherein the formulation further comprises sucrose.

22. The method of claim 21, wherein the formulation further comprises sucrose at a concentration of about 234 mM.

23. The method of any preceding claim, wherein the formulation is formulated to a pH of about 6.0.

24. The method of any preceding claim, wherein the formulation further comprises polysorbate-80.

25. The method of any preceding claim wherein the therapeutic protein is an antigen binding polypeptide.

26. The method of any preceding claim wherein the antigen binding polypeptide is an antibody.

27. The method of any preceding claim wherein the antigen binding polypeptide is an immunoglobulin single variable domain.

28. The method of claim 25 where the antigen binding polypeptide binds to interleukin 5 (IL5).

29. The method of claim 28, wherein the antigen binding polypeptide is an anti-IL5 antibody.

30. The method of claim 29, wherein the anti-IL5 antibody comprises a heavy chain comprising SEQ ID NO:1 and a light chain comprising SEQ ID NO:2.

31. The method of any preceding claim wherein the therapeutic protein is present at a concentration of at least about 150 mg/ml, at least about 175 mg/ml, at least about 200 mg/ml, at least about 225 mg/ml, at least about 250 mg/ml, at least about 275 mg/ml, or at least about 300 mg/ml.

32. The method of any preceding claim wherein the therapeutic protein is present at a concentration of at least about 150 mg/ml to about 300 mg/ml.

33. The method of any preceding claim wherein the formulation is a reconstituted formulation.

34. The method of any preceding claim wherein the formulation is a liquid pharmaceutical formulation.

35. The method of any preceding claim wherein the formulation is suitable for parenteral administration.

36. The method of any preceding claim wherein the formulation comprises about 10 mM to about 50 mM citrate.

37. The method of claim 36 wherein the formulation comprises about 20 mM citrate.

38. A stable formulation produced by the method of any preceding claim.

39. An article of manufacture comprising a container containing the formulation of claim 38.

40. The article of manufacture of claim 39 further comprising directions for administration of the formulation.

Figure 1

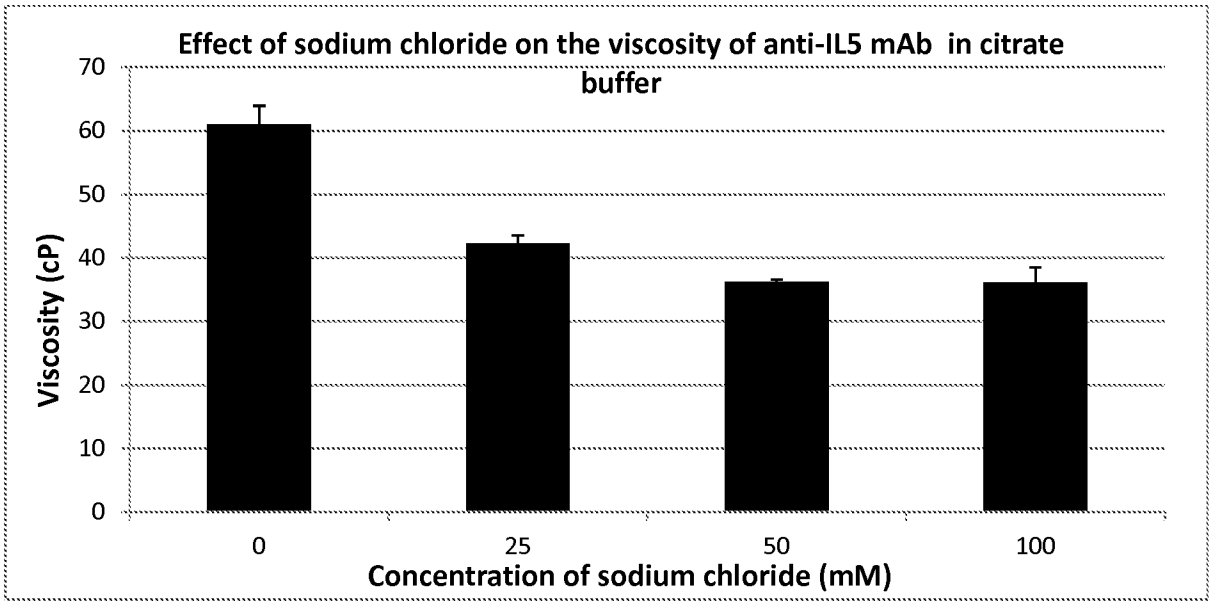

Effect of sodium chloride on the viscosity of anti-IL5 mAb in citrate buffer
Viscosity (cP)
70
60
50
40
30
20
10
0
0
25
50
100
Concentration of sodium chloride (mM)

Figure 2

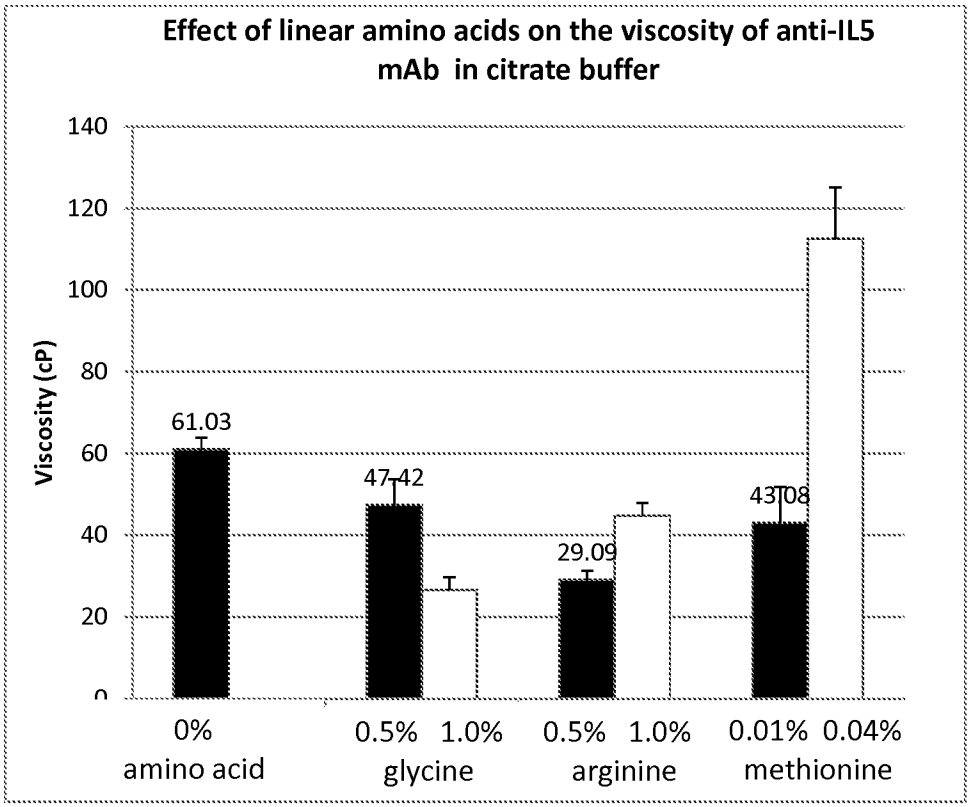
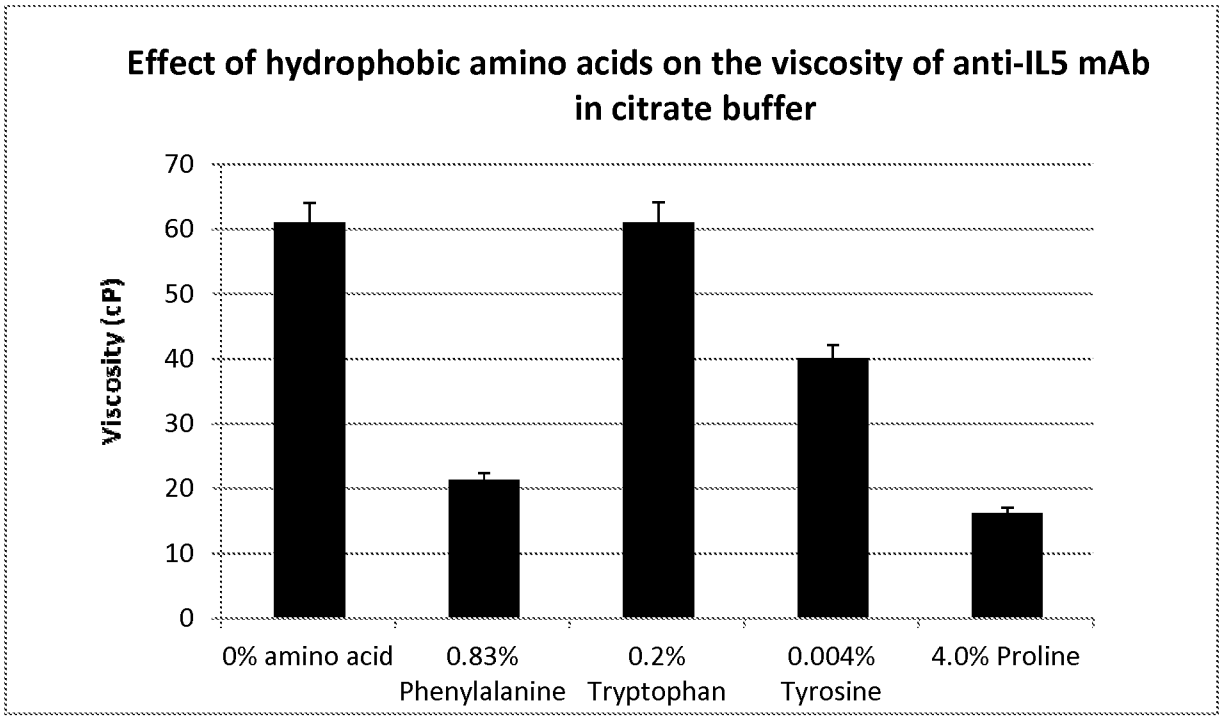

Effect of linear amino acids on the viscosity of anti-IL5 mAb in citrate buffer
Viscosity (cP)
140
120
100
80
60
40
20
0
61.03
47.42
29.09
43.08
0%
amino acid
0.5%
1.0%
glycine
0.5%
1.0%
arginine
0.01%
0.04%
methionine

Figure 3


Effect of hydrophobic amino acids on the viscosity of anti-IL5 mAb in citrate buffer
Viscosity (cP)
70
60
50
40
30
20
10
0
0% amino acid
0.83% Phenylalanine
0.2% Tryptophan
0.004% Tyrosine
4.0% Proline

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/032462

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/00 (2012.01)
USPC - 424/130.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 33/00, 39/06, 39/395; C07K 16/00 (2012.01)
USPC - 424/130.1, 201.1, 489; 530/387.1

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)
PatBase, Google Patents, Google, PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/0160014 A1 (WARNE et al) 03 July 2008 (03.07.2008) entire document	1-3, 5-7, 9
Y		10, 11, 13-15, 17-19
Y	US 2005/0053666 A1 (TZANNIS et al) 10 March 2005 (10.03.2005) entire document	10, 11, 13-15, 17-19
Y	US 2006/0127414 A1 (MAYERESSE et al) 15 June 2006 (15.06.2006) entire document	13-15
Y	US 2010/0297106 A1 (SLOEY et al) 25 November 2010 (25.11.2010) entire document	19
A	US 7,666,413 B2 (LIU et al) 23 February 2010 (23.02.2010) entire document	1-3, 5-7, 9-11, 13-15, 17-19

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
08 June 2012	06 JUL 2012

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

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/032462

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

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

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

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

3. ☒ Claims Nos.: 4, 8, 12, 16, 20-40
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:

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

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

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

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.