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(54) **NOVEL FORMULATIONS FOR ANTIBODIES**

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ABSTRACT

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§ 371 (c)(1),

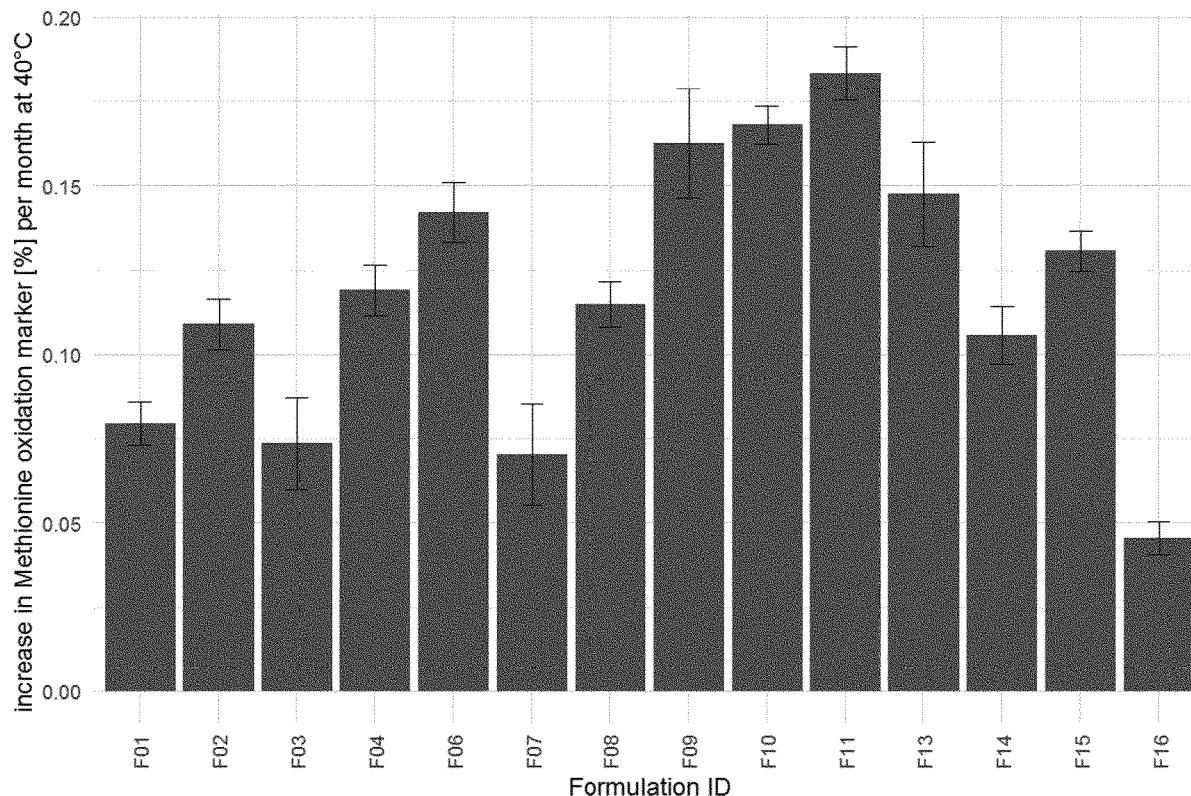
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The present invention relates in general to a stable, preferably liquid, pharmaceutical formulation comprising an antibody, a buffer, a surfactant, a stabilizer selected from the group consisting of sugars and sugar alcohols, and further optionally a chelator and/or oxygen-scavenger. Furthermore, the present invention relates to an article of manufacture comprising a container and a stable pharmaceutical formulation of the invention.

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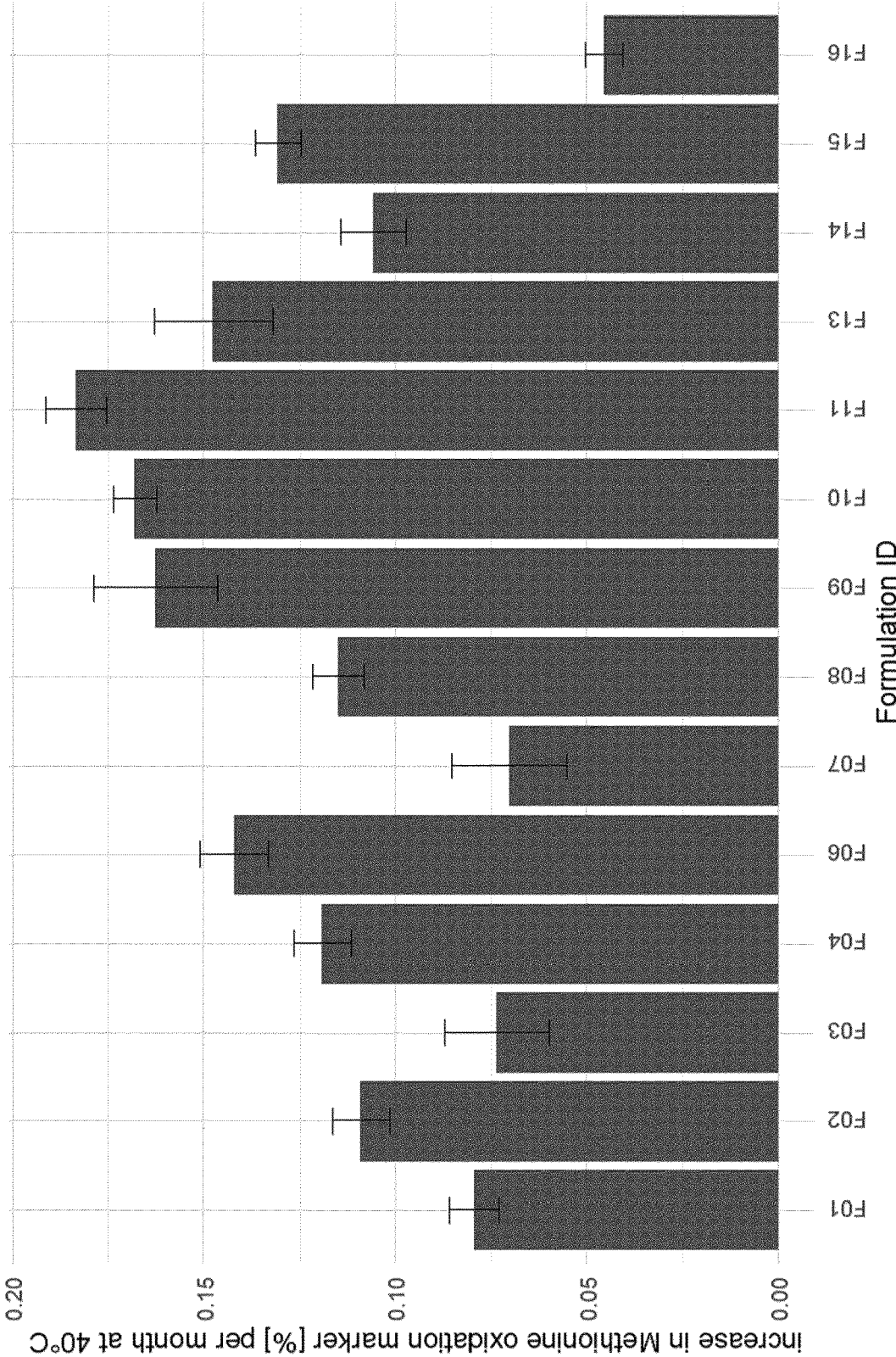


Fig.1

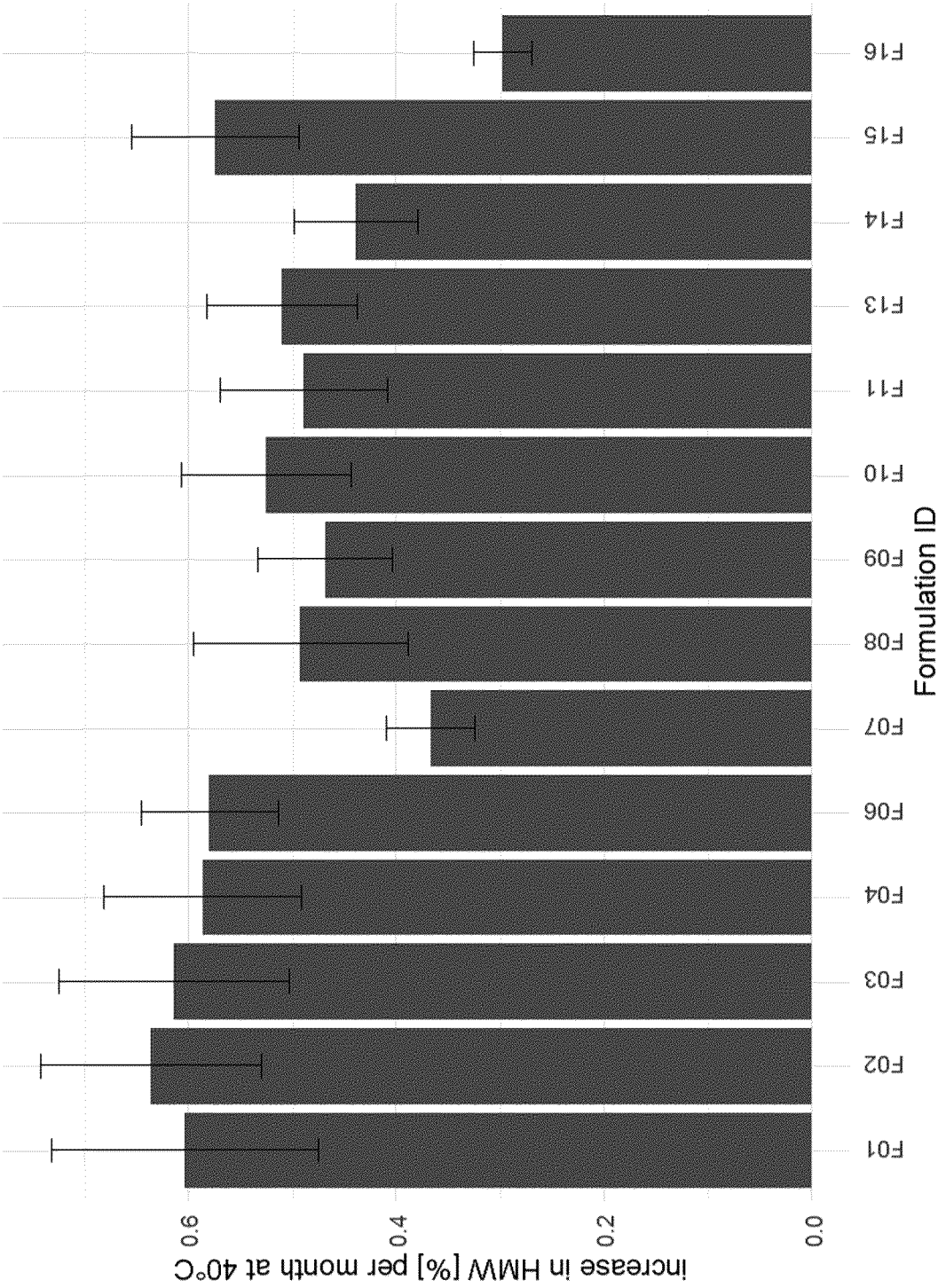


Fig.2

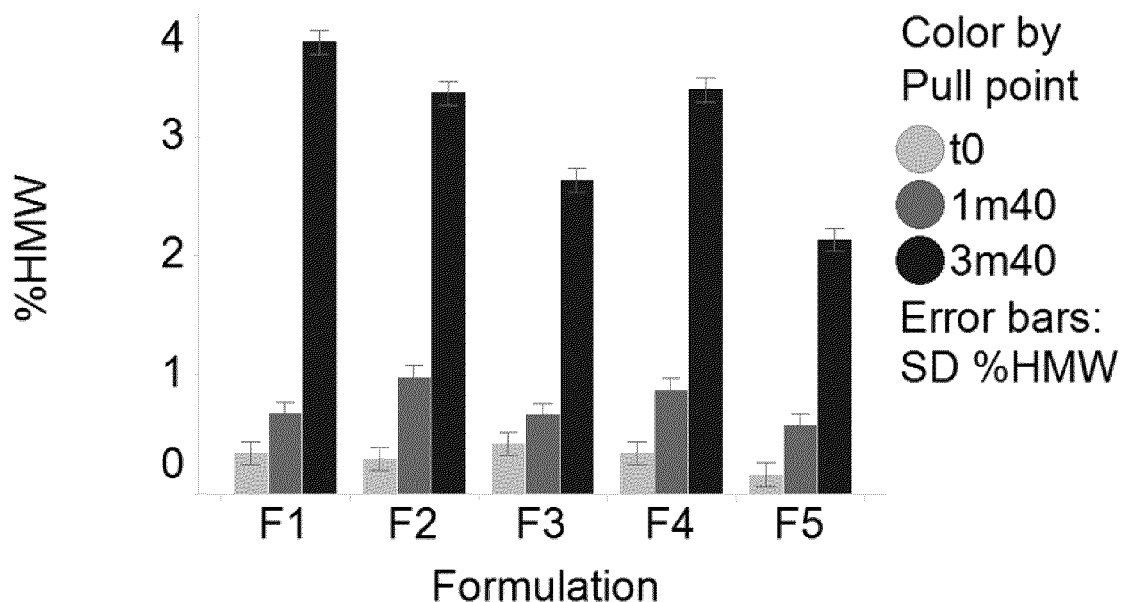


Fig.3

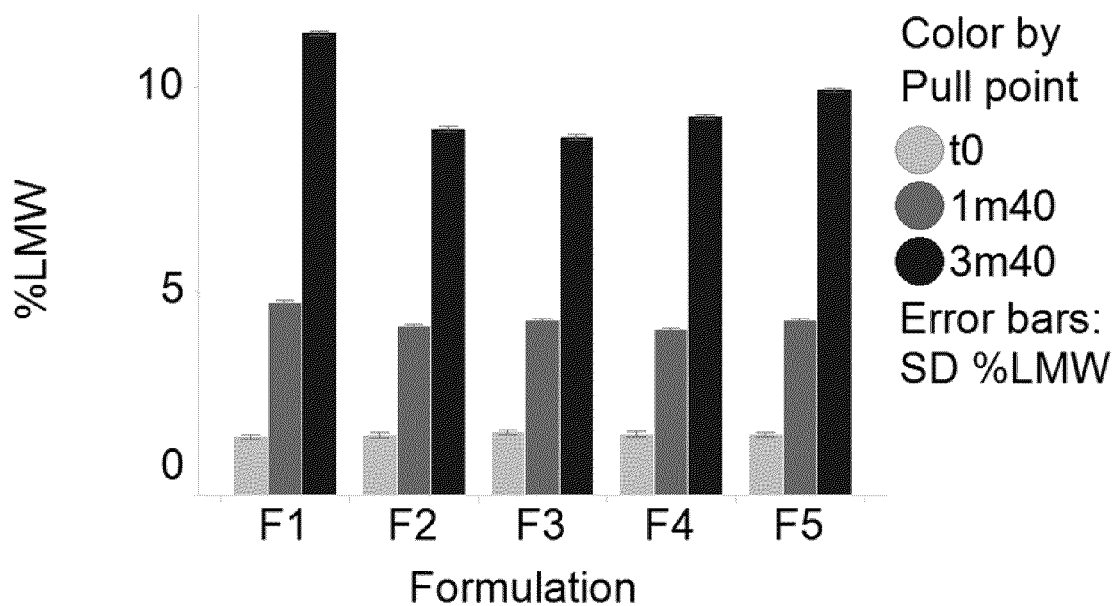


Fig.4

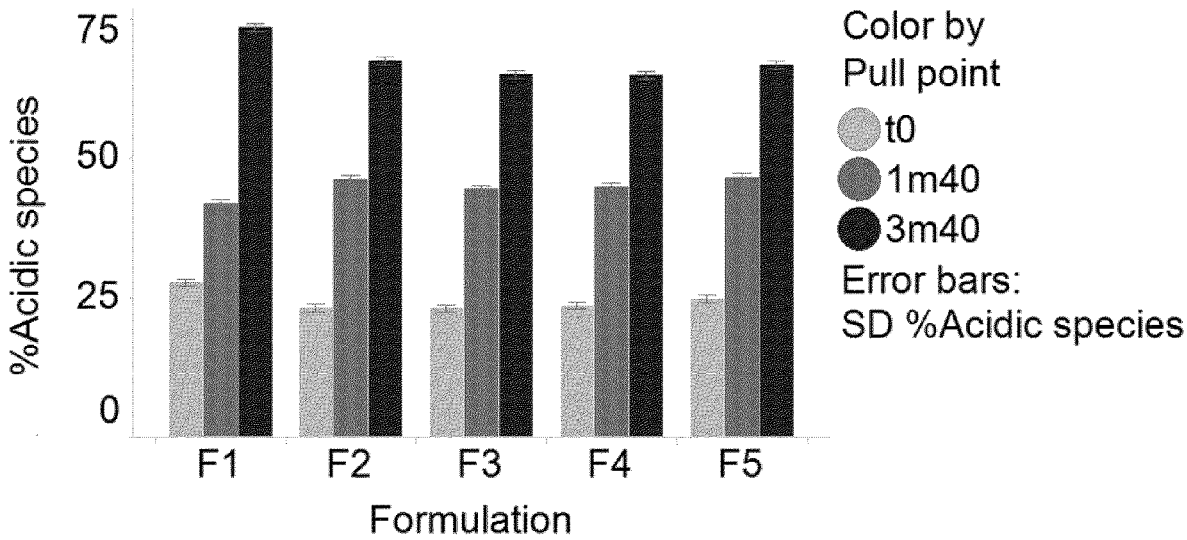


Fig.5

NOVEL FORMULATIONS FOR ANTIBODIES

[0001] The present invention relates to a stable, preferably liquid, pharmaceutical formulation comprising an antibody, a buffer, a surfactant, a stabilizer selected from the group consisting of sugars and sugar alcohols, and further optionally a chelator and/or oxygen-scavenger. Furthermore, the present invention relates to an article of manufacture comprising a container and a stable pharmaceutical formulation of the invention

[0002] Advances in biotechnology have made it possible to produce a variety of proteins for pharmaceutical applications. Because proteins are large and complex (i.e., possessing multiple functional groups in addition to complex three-dimensional structures), the formulation of such proteins poses special problems. For a protein to remain biologically active, a formulation must preserve the conformational integrity of at least a core sequence of the protein's amino acids, while at the same time protecting the protein's multiple functional groups from degradation. Proteins may suffer from a lack of stability, and monoclonal and polyclonal antibodies in particular may be relatively unstable.

[0003] Among the many problems in technical development of biotherapeutic antibodies, chemical modifications during storage pose a key challenge. Specifically, oxidation of methionine in biotherapeutic drug substances such as antibodies is of great concern since it may alter critical properties as efficacy (e.g. reduced target binding of an antibody), or safety (e.g. greater immunogenic potential due to increased aggregation). Antibodies are exposed to oxidative stress due to oxygen present in the headspace, metal ion leachables stemming from the primary packaging material or processing materials, or from trace impurities of excipients used in the formulation. Frequently, marked oxidation behaviour of antibodies can be observed. Oxidation of antibodies in turn may correlate with increased aggregation rates during storage in solution. This correlation highlights the need to establish tight control of oxidation in antibody formulations in order to prevent deterioration of key quality attributes of any given antibody drug product.

[0004] Although there are various antibody formulations on the market, there is still an urgent need to develop formulations, which give rise to steady, therapeutically effective blood levels of the antibody formulations over an extended period of time in a stable and convenient form. The problem to be solved by the present invention was thus to provide further antibody formulations with satisfactory stability, in particular in terms of low oxidation and/or aggregation rates.

[0005] This problem is solved by the subject-matter as set forth below and in the appended claims.

[0006] In a first aspect the present invention relates to a stable, preferably liquid, pharmaceutical formulation comprising an antibody, a buffer, a surfactant, a stabilizer selected from the group of sugars and sugar alcohols, in particular sorbitol, and, optionally, a chelator and/or oxygen-scavenger.

[0007] A "stable" formulation according to the present invention is a formulation in which the antibody retains its physical stability and/or chemical stability and/or biological activity upon storage. Preferably, the antibody essentially retains its physical and chemical stability, as well as its biological activity upon storage. The storage period is generally selected based on the intended shelf-life of the formulation. Various analytical techniques for measuring pro-

tein stability are available in the art. Stability can be evaluated qualitatively and/or quantitatively in a variety of different ways, including evaluation of aggregate formation (for example using size exclusion chromatography, by measuring turbidity, and/or by visual inspection); by assessing charge heterogeneity using cation exchange chromatography, image capillary isoelectric focusing (icIEF) or capillary zone electrophoresis; amino-terminal or carboxy-terminal sequence analysis; mass spectrometric analysis; SDS-PAGE analysis to compare reduced and intact antibody; peptide map (for example tryptic or LYS-C) analysis; evaluating biological activity or antigen binding function of the antibody; etc. Instability may involve any one or more of: aggregation, deamidation (e.g. Asn deamidation), oxidation (e.g. Met oxidation), isomerization (e.g. Asp isomerization), clipping/hydrolysis/fragmentation (e.g. hinge region fragmentation), succinimide formation, unpaired cysteine(s), N-terminal extension, C-terminal processing, glycosylation differences, etc. According to the present invention, a stable formulation is in particular a formulation which shows a low or negligible increase in methionine oxidation at surrogate site methionine within sequence GNVFSCSVMHEALHNHYTQK (which can be found in all IgG subtypes, SEQ ID NO:1). A low or negligible increase is an increase of less than 0.50% per month, preferably less than 0.20% per month, more preferably less than 0.15% per month, even more preferably less than 0.10% per month, after storage at 40° C. for 12 weeks. Alternatively, or in addition, the stable formulation of the invention exhibits an increase in high molecular weight species as determined by size exclusion chromatography of less than 1.3% per month, more preferably of less than 1.2% per month, more preferably of less than 1.1% per month, more preferably of less than 1.0% per month, more preferably of less than 0.8% per month, even more preferably less than 0.6% per month, and most preferably of less than 0.4% per month after storage at 40° C. for 12 weeks. Exemplary and preferred methods of assessing stability in terms of increase in methionine oxidation and increase in high molecular weight species are disclosed in the examples section of this application.

[0008] The antibody contained in the inventive formulation can be any antibody of interest. In certain embodiments, the antibody is a monoclonal antibody. In certain embodiments, the monoclonal antibody is a full-length antibody (e.g., IgG1, IgG4, etc.). Most preferably, the antibody is an IgG4 antibody. In certain embodiments, the monoclonal antibody is an antibody fragment (e.g., comprising an antigen binding region). For example, the antibody fragment is a Fab or F(ab')₂ fragment. In certain embodiments, the monoclonal antibody is a humanized antibody. In certain embodiments, the monoclonal antibody is susceptible to aggregation. The antibody may for example be directed against the following: tumour antigen CA-125, TNF- α , CD52, A β , CD25, B-lymphocyte stimulator (BLyS), vascular endothelial growth factor A (VEGF-A), IL-12, IL-23, IL-1 β , epithelial cell adhesion molecule (EpCAM), epidermal growth factor receptor (EGFR; ErbB-1; HER1 in humans), RANK ligand (RANKL), CD11a, CD33, programmed cell death protein 1 (PD-1), carbonic anhydrase IX (CA-IX), CD20, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), CD23, IL-5, RSV glycoprotein F, CD3, fungal Hsp90, alpha-4 integrin, IgE Fc region, receptor tyrosine-protein kinase erbB-2 (HER2), or IL-6 receptor. In a preferred embodiment, the antibody comprises a light chain

variable region comprising a complementarity determining region 1 (CDR1) according to SEQ ID NO:2, a CDR2 according to SEQ ID NO:3, and a CDR3 according to SEQ ID NO:4, and/or comprises a heavy chain variable region comprising a CDR1 according to SEQ ID NO:5, a CDR2 comprising according to SEQ ID NO:6, and a CDR3 comprising according to SEQ ID NO:7. Preferably, and antibody comprising these CDRs according to SEQ ID NO: 2 to 7 is an antibody which comprises a light chain according to SEQ ID NO:8 and a heavy chain according to SEQ ID NO:9. In an equally preferred embodiment, the antibody comprises a light chain variable region comprising a complementarity determining region 1 (CDR1) according to SEQ ID NO:10, a CDR2 according to SEQ ID NO:11, and a CDR3 according to SEQ ID NO:12, and/or comprises a heavy chain variable region comprising a CDR1 according to SEQ ID NO:13, a CDR2 comprising according to SEQ ID NO:14, and a CDR3 comprising according to SEQ ID NO:15. Preferably, and antibody comprising these CDRs according to SEQ ID NO: 10 to 15 is an antibody comprising a light chain according to SEQ ID NO:16 and a heavy chain according to SEQ ID NO:17.

[0009] The antibody is preferably present in a concentration of up to about 80 mg/ml, preferably in the range of about 5 to about 50 mg/ml, more preferably in the range of 5 to 30 mg/ml, even more preferably in the range of about 10 mg/ml to 25 mg/ml. A particularly preferred concentration for an antibody comprising CDRs according to SEQ ID NO: 2 to 7, such as an antibody comprising a light chain according to SEQ ID NO:8 and a heavy chain according to SEQ ID NO:9 is about 10 mg/ml. A particularly preferred concentration for an antibody comprising CDRs according to SEQ ID NO: 10 to 15, such as an antibody comprising a light chain according to SEQ ID NO:16 and a heavy chain according to SEQ ID NO:17 is about 25 mg/ml.

[0010] The inventive stable antibody formulation comprises a buffer. The buffer can be any buffer suitable for storage of the antibody contained in the formulation. Preferably the buffer is selected from the group consisting of an acetate buffer, adipate buffer, histidine buffer, citrate buffer or succinate buffer. An "acetate buffer" is a buffer comprising acetate ions. Preferably, an acetate buffer does not comprise adipate, histidine, citrate and/or succinate ions. Examples of adipate buffers include sodium adipate, ammonium adipate, calcium adipate, magnesium adipate and potassium adipate solutions, with sodium adipate being particularly preferred. The adipate buffer has preferably a pH between about pH 5.0 to 6.5, preferably about pH 5.5. An "adipate buffer" is a buffer comprising adipate ions. Preferably, an adipate buffer does not comprise acetate, histidine, citrate and/or succinate ions. Examples of histidine buffers include histidine chloride, histidine acetate, histidine phosphate, and histidine sulfate solutions, with histidine chloride (His/HCl) being particularly preferred. The histidine buffer or histidine-HCl buffer has preferably a pH between about pH 5.0 to 6.5, about pH 5.5 to 6.2, or about pH 6.0. A "histidine buffer" is a buffer comprising histidine ions. Preferably, a histidine buffer does not comprise acetate, adipate, citrate and/or succinate ions. Examples of histidine buffers include histidine chloride, histidine acetate, histidine phosphate, and histidine sulfate solutions, with histidine chloride (His/HCl) being particularly preferred. The histidine buffer or histidine-HCl buffer has preferably a pH between about pH 5.0 to 6.5, about pH 5.5 to 6.2, or about pH 6.0. A "citrate buffer" is a buffer comprising citrate ions. Preferably, a citrate buffer does not comprise acetate, adipate, histidine and/or succinate ions.

Examples of citrate buffers include sodium citrate, ammonium citrate, calcium citrate, magnesium citrate and potassium citrate solutions. The citrate buffer has preferably a pH between about pH 5.0 to 6.5, about pH 5.5 to 6.2, or about pH 6.0. A "succinate buffer" is a buffer comprising succinate ions. Preferably, a succinate buffer does not comprise acetate, adipate, histidine and/or citrate ions. Examples of succinate buffers include sodium succinate, ammonium succinate, calcium succinate, magnesium succinate and potassium succinate solutions. The succinate buffer has preferably a pH between about pH 5.0 to 6.5, about pH 5.5 to 6.2, or about pH or about pH 6.0. In preferred embodiments of the invention, the buffer of the formulation of the present invention is a sodium succinate buffer (preferably at pH 6.0), a histidine-HCl buffer (preferably at pH 6.0) or a sodium adipate buffer (preferably at pH 6.0). pH is measured using standard glass bulb pH meter. Unless otherwise indicated, the pH values and ranges defined herein for formulations of the present invention reflect the pH at the respective storage temperature of the formulation, which is in the range of 2-8° C. In buffers comprising an antibody comprising CDRs according to SEQ ID NO: 2 to 7, such as an antibody comprising a light chain according to SEQ ID NO:8 and a heavy chain according to SEQ ID NO:9, the buffer has preferably a pH of 6.0. In buffers comprising an antibody comprising CDRs according to SEQ ID NO: 10 to 15, such as an antibody comprising a light chain according to SEQ ID NO:16 and a heavy chain according to SEQ ID NO:17, the buffer has preferably a pH of 5.5. In preferred embodiments, in particular for formulations comprising an antibody comprising CDRs according to SEQ ID NO: 2 to 7, such as an antibody comprising a light chain according to SEQ ID NO:8 and a heavy chain according to SEQ ID NO:9, the buffer is selected from the group consisting of adipate buffer, histidine buffer, and succinate buffer. In other preferred embodiments, in particular for formulations comprising an antibody comprising CDRs according to SEQ ID NO: 10 to 15, such as an antibody comprising a light chain according to SEQ ID NO:16 and a heavy chain according to SEQ ID NO:17, the buffer is selected from the group consisting of acetate buffer, adipate buffer, and succinate buffer. The concentration of the buffer in the inventive formulation is preferably in the range of 5 to 50 mM, even more preferably in the range of 10 to 20 mM. Most preferably, the concentration of the buffer is 10 mM. Preferred buffers for the formulation of the present invention, in particular for formulations comprising an antibody comprising CDRs according to SEQ ID NO: 2 to 7, such as an antibody comprising a light chain according to SEQ ID NO:8 and a heavy chain according to SEQ ID NO:9, are a 20 mM sodium succinate buffer, a 10 mM histidine/HCl buffer as well as a 10 mM sodium adipate buffer. Other preferred buffers for the formulation of the present invention, in particular for formulations comprising an antibody comprising CDRs according to SEQ ID NO: 10 to 15, such as an antibody comprising a light chain according to SEQ ID NO:16 and a heavy chain according to SEQ ID NO:17, are a 10 mM succinate buffer, a 10 mM acetate buffer as well as a 10 mM adipate buffer.

[0011] Aside of the buffer, the inventive formulation also comprises a surfactant. The surfactant can be in principle any (non-ionic) surfactant suitable for antibody formulation, but it is preferably, selected from the group of polysorbate 20, polysorbate 80, a poloxamer (such as poloxamer 188)

and combinations thereof. Most preferably, the surfactant is polysorbate 20 or polysorbate 80. Typically, but not necessarily, the surfactant, and in particular polysorbate 20 or polysorbate 80, will be present in a concentration of about 0.01% to about 0.1% w/v. Formulations of the present invention may for example comprise about 0.1 mg/ml, about 0.2 mg/ml, about 0.3 mg/ml, about 0.4 mg/ml, about 0.5 mg/ml, about 0.6 mg/ml, about 0.7 mg/ml, about 0.8 mg/ml, about 0.9 mg/ml or about 1 mg/ml. In preferred embodiments of the invention, the surfactant is chosen from polysorbate 20 and polysorbate 80, and is present in a concentration of about 0.1 mg/ml to about 0.3 mg/ml. More preferably, the concentration of polysorbate 20 and polysorbate 80 is about 0.2 mg/ml. Most preferably, the surfactant is polysorbate 80 in a concentration of about 0.2 mg/ml.

[0012] A further component of the inventive formulation is a stabilizer selected from the group of sugars and sugar alcohols. Examples of sugars are lactose, fructose, maltose, trehalose and sucrose. Most preferably, the sugar is sucrose. Preferred examples of sugar alcohols are arabinol, mannitol and sorbitol. Most preferably, in particular if the formulation of the invention is a liquid formulation, sorbitol is a component of the inventive formulation. Sorbitol can help to suppress for example antibody aggregation, in particular in the course of freeze/thaw cycles. Typically, sorbitol is present in a concentration no greater than approximately 1 M in the liquid formulation of the invention. In some embodiments, sorbitol is present in a concentration no greater than approximately 300 mM. In many embodiments, the concentration of sorbitol will be in the range of 100 to 350 mM, more preferably in the range of 150 to 300 mM, even more preferably in the range of 165 mM to 285 mM sorbitol. Preferred sorbitol concentrations are for example 165 mM, 215 mM, 265 mM and 285 mM sorbitol. In some embodiments of the invention, the stable (preferably liquid) formulation of the invention does not contain mannitol. If the inventive formulation contains sucrose, it is preferably used in the range of 100 to 300 mM sucrose, preferably in the range of 140 to 210 mM sucrose. The formulation may also comprise a combination of sucrose and 2-hydroxypropyl-beta-cyclodextrin. In some cases, in particular if the inventive formulation comprises an antibody comprising CDRs according to SEQ ID NO: 10 to 15, such as an antibody comprising a light chain according to SEQ ID NO:16 and a heavy chain according to SEQ ID NO:17, the formulation may comprise a sugar or a sugar alcohol, such as sorbitol or a combination of sucrose and 2-hydroxypropyl-beta-cyclodextrin.

[0013] A further, optional, component of the inventive formulation is a chelator and/or oxygen-scavenger or combinations of both. While said component is optional, it is nonetheless preferred if said optional component is present in the inventive formulation. Typical chelators are citrate, diethylenetriaminepentaacetic acid (DTPA), and ethylenediaminetetraacetic acid (EDTA), with the latter two being more preferred in the context of the present invention, and EDTA being the most preferred chelator of the invention. Examples for oxygen scavengers are methionine, sodium thiosulfate, catalase, or platinum, wherein methionine is the preferred oxygen scavenger for the present invention. Moreover, it is preferred if the inventive formulation comprises only one of chelator and oxygen-scavenger, not both. In case the inventive formulation comprises a chelator, such as DTPA or EDTA, the such chelator may be present in a

concentration in the range of 5 to 150 μ M, preferably in the range of 20 to 100 μ M. Particularly preferred concentrations are 20 μ M, 50 μ M, and 100 μ M. Most preferred is a chelator concentration of 20 μ M. In case the inventive formulation comprises an oxygen scavenger such as methionine, then such scavenger is preferably present in a concentration of about 100 μ M to 10 mM. A particularly preferred concentration of methionine is about 6702 μ M. Instead of using a chelator/and or oxygen scavenger, one may also consider to flush the headspace of the container containing the inventive formulation with an inert gas, e.g. with nitrogen, in order to displace oxygen and to minimize oxidation.

[0014] Finally, the inventive formulation may optionally also comprise salt such as sodium sulphate or sodium chloride or combinations thereof. Sodium chloride can reduce the viscosity of a formulation. The inventive formulation may—without being limited thereto—comprise 10 to 100 mM salt, for example 10 to 20 mM sodium sulphate, in particular 16 mM sodium sulphate, or may comprise 25 to 75 mM sodium chloride, in particular 40 to 60 mM sodium chloride, in particular 50 mM sodium chloride. Using a salt is in particular an option in cases where the inventive formulation comprises an antibody comprising CDRs according to SEQ ID NO: 2 to 7, such as an antibody comprising a light chain according to SEQ ID NO:8 and a heavy chain according to SEQ ID NO:9.

[0015] The stable formulation according to the present invention is preferably a liquid formulation. However, in some embodiments, in particular where the formulation does not comprise sorbitol, (but instead, for example, a sugar such as sucrose or a combination of sucrose and 2-hydroxypropyl-beta-cyclodextrin), the formulation may also be present in lyophilized form.

[0016] Liquid formulations specifically contemplated by the inventors of the present application, in particular in cases where the inventive formulation comprises an antibody comprising CDRs according to SEQ ID NO: 2 to 7, such as an antibody comprising a light chain according to SEQ ID NO:8 and a heavy chain according to SEQ ID NO:9 comprise a) a succinate buffer, EDTA, sorbitol and polysorbate 80, b) a histidine buffer, sodium chloride, EDTA, sorbitol and polysorbate 20, or c) a sodium adipate buffer, methionine, sorbitol and polysorbate 80. For instance, inventive formulations may comprise a) 20 mM sodium succinate buffer, pH 6.0, 265 mM sorbitol, 20 μ M or 50 μ M EDTA, and 0.2 mg/ml polysorbate 80, and no salt, i.e. sodium chloride and/or sodium sulphate, in particular no sodium chloride, b) 10 mM histidine/HCl buffer, pH 6.0, 50 mM sodium chloride, 215 mM sorbitol, 20 μ M or 50 μ M EDTA, and 0.2 mg/ml polysorbate 20, c) 10 mM histidine/HCl buffer, pH 6.0, 50 mM sodium chloride, 165 mM sorbitol, 20 μ M or 50 μ M EDTA, and 0.2 mg/ml polysorbate 20, or d) 10 mM sodium adipate buffer, pH 6.0, 265 mM or 285 mM sorbitol, 6702 μ M methionine and 0.2 mg/ml polysorbate 80, and also no salt, i.e. no sodium chloride and/or sodium sulphate, in particular no sodium chloride.

[0017] In other embodiments, in particular in cases where the inventive formulation comprises an antibody comprising CDRs according to SEQ ID NO: 10 to 15, such as an antibody comprising a light chain according to SEQ ID NO:16 and a heavy chain according to SEQ ID NO:17, the inventors specifically contemplate the following buffers: a) a succinate buffer, sucrose and polysorbate 80, b) an acetate buffer, sucrose and polysorbate 80, and c) an adipate buffer,

sucrose and polysorbate 80 (the sucrose in these formulations can be replaced by sorbitol). For instance, such formulations may comprise a) a 10 mM succinate buffer, 204.5 mM sucrose and 0.2 mg/ml polysorbate 80, b) a 10 mM acetate buffer, 204.5 mM sucrose and 0.2 mg/ml polysorbate 80, c) a 10 mM adipate buffer, 204.5 mM sucrose and 0.2 mg/ml polysorbate 80 or d) 10 mM succinate buffer, 204.5 mM sorbitol and 0.2 mg/ml polysorbate 80. In some of these embodiments, the formulation may comprise a combination of sucrose and 2 hydroxypropyl-beta-cyclodextrin (e.g. Kleptose®), e.g. about 146 mM sucrose and about 22 mM 2 hydroxypropyl-beta-cyclodextrin or about 160 mM sucrose and about 40 mM 2 hydroxypropyl-beta-cyclodextrin.

[0018] In a further aspect, the present invention relates to an article of manufacture comprising a container, a stable, preferably liquid, pharmaceutical formulation according to the present invention and instructions for its use. The article of manufacture may in particular be a container such as a pre-filled syringe, such as a filled glass syringe, or a glass vial.

[0019] Finally, the present invention relates to a stable pharmaceutical formulation according to the present invention, in particular a stable liquid pharmaceutical formulation according to the present invention, for use in a method of treating a disease of the human or animal body, in particular wherein the disease is cancer. The cancer may be any type of cancer for which the antibody of the inventive formulation is therapeutically effective. For instance, the cancer may be selected from the group consisting of lung, colon, bladder, cervix, skin, ovary, endometrial, gastric, head and neck, breast, melanoma, pancreas, kidney, prostate, oesophagus, stomach or bile duct cancer. Similarly, the cancer may for example be selected from the group consisting of renal carcinoma (RCC), classical Hodgkin lymphoma (cHL), non-Hodgkin's lymphoma (NHL), non-small cell lung carcinoma (NSCLC), squamous cell cancer of the head and neck (SCCHN or HNSCC, respectively), urothelial carcinoma, glioma, colorectal cancer, chronic lymphocytic leukaemia (CLL), follicular lymphoma, and breast carcinoma.

[0020] The term "comprising", as used herein, shall not be construed as being limited to the meaning "consisting of" (i.e. excluding the presence of additional other matter). Rather, "comprising" implies that optionally additional matter, features or steps may be present. The term "comprising" encompasses as particularly envisioned embodiments falling within its scope "consisting of" (i.e. excluding the presence of additional other matter) and "comprising but not consisting of" (i.e. requiring the presence of additional other matter, features or steps), with the former being more preferred.

[0021] The use of the word "a" or "an", when used herein, may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

FIGURES

[0022] In the following a brief description of the appended figures will be given. The figures are intended to illustrate the present invention in more detail. However, it is not intended to limit the scope of the invention to only these specifically illustrated embodiments.

[0023] FIG. 1: illustrates the methionine oxidation rate per month for an antibody heavy chain (SEQ ID NO:9); total incubation time: 12 weeks at 40° C. Error bars indicate standard errors of linear regression for 4 data points.

[0024] FIG. 2: illustrates the formation of high molecular weight species of an antibody comprising an antibody heavy chain according to SEQ ID NO:9 and a light chain according to SEQ ID NO:8); per month of incubation at 40° C. Total incubation time 12 weeks for 40° C. Antibody as determined by size exclusion chromatography. Error bars indicate fit error from linear regression of 4 data points.

[0025] FIG. 3: illustrates the increase of aggregates over 3 months storage at 40° C. in different formulations for an antibody comprising an antibody heavy chain according to SEQ ID NO:17 and a light chain according to SEQ ID NO:16.

[0026] FIG. 4: illustrates the increase of acidic species over 3 months storage at 40° C. in different formulations for an antibody comprising an antibody heavy chain according to SEQ ID NO:17 and a light chain according to SEQ ID NO:16.

[0027] FIG. 5: illustrates the increase of degradation products over 3 months storage at 40° C. in different formulations for an antibody comprising an antibody heavy chain according to SEQ ID NO:17 and a light chain according to SEQ ID NO:16.

EXAMPLES

[0028] In the following, specific examples illustrating various embodiments and aspects of the invention are presented. However, the present invention shall not be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become readily apparent to those skilled in the art from the foregoing description, accompanying figures and the examples below. All such modifications fall within the scope of the appended claims.

Example 1: Analysis of Oxidation of an Antibody in the Presence of DTPA, EDTA or Oxygen Scavenger Methionine

[0029] The effect of various formulations on methionine oxidation rate per month of an antibody heavy chain (SEQ ID NO:9) after storage for 12 weeks at a storage temperature of 40° C. was tested. For this purpose, the following experimental set-up was used:

[0030] 10 mL of sterile filtered sample solution was filled into 25R glass vials (hydrolytic glass type I) and closed with a FluroTec® coated stopper and a crimp cap. Filled vials were stored inverted and incubated until the respective pull points at 40±2° C./75±5% RH. Oxidation was determined using a reduced peptide mapping approach. Samples were denatured using guanidinium hydrochloride, followed by reduction with dithiothreitol and alkylation using iodoacetamide. Peptide fragments were generated using lysyl-endopeptidase C (Lys-C) and subsequently analysed using RP-HPLC FLD. Relative quantification was performed on one methionine sulfoxide containing reporter peptide derived from the IgG C-terminus (Met 421) as percentage of the sum of its native peptide and the oxidised variant. Note that methionine oxidation results in formation of methionine sulfoxide diastereomers, resulting in two potential reporter peptides. Here, only one of them was chosen as reporter peptide for quantification of methionine oxidation.

[0031] Formulations to be analysed were selected from the following list:

molecular weight species per month after storage at a storage temperature of 40° C. for 12 weeks.

TABLE 1

List of formulations, all formulations contain protein at 10 mg/mL							
Formulation ID	Buffer type	Buffer pH	Buffer conc. (mM)	Salt	Sugar alcohol	Chelator	Surfactant
F01	Sodium Citrate	6.0	20.0	50 mM NaCl	165 mM mannitol	20 µM DTPA	0.2 mg/mL PS80
F02	Sodium Citrate	6.0	10.0	16 mM Na ₂ SO ₄	165 mM sorbitol	50 µM EDTA	0.2 mg/mL PS80
F03	Sodium Succinate	6.0	20.0	50 mM NaCl	165 mM sorbitol	20 µM DTPA	0.2 mg/mL PS80
F04	Sodium Succinate	6.0	20.0	50 mM NaCl	165 mM sorbitol	50 µM EDTA	0.2 mg/mL PS80
F05	Sodium Succinate	6.0	20.0	50 mM NaCl	165 mM sorbitol	None	0.2 mg/mL PS80
F06a	Sodium Succinate	6.0	20.0	none	265 mM sorbitol	20 µM EDTA	0.2 mg/mL PS80
F06	Sodium Succinate	6.0	20.0	none	265 mM sorbitol	50 µM EDTA	0.2 mg/mL PS80
F07	Sodium Succinate	6.0	20.0	50 mM NaCl	165 mM sorbitol	6702 µM methionine	0.2 mg/mL PS80
F08	His/HCl	6.0	10.0	50 mM NaCl	165 mM sorbitol	20 µM DTPA	0.2 mg/mL PS80
F09	His/HCl	6.0	10.0	50 mM NaCl	165 mM sorbitol	100 µM EDTA	0.2 mg/mL PS80
F10a	His/HCl	6.0	10.0	50 mM NaCl	215 mM sorbitol	20 µM EDTA	0.2 mg/mL PS20
F10	His/HCl	6.0	10.0	50 mM NaCl	165 mM sorbitol	50 µM EDTA	0.2 mg/mL PS20
F11	His/HCl	6.0	10.0	50 mM NaCl	165 mM sorbitol	20 µM EDTA	0.2 mg/mL PS80
F12	His/HCl	6.0	10.0	50 mM NaCl	165 mM sorbitol	None	0.2 mg/mL PS80
F13	His/HCl	6.0	10.0	50 mM NaCl	165 mM sorbitol	6702 µM methionine	0.2 mg/mL PS80
F14	His/HCl	6.0	10.0	None	265 mM sorbitol	6702 µM methionine	0.2 mg/mL PS80
F15	Sodium adipate	6.0	20.0	50 mM NaCl	165 mM sorbitol	50 µM EDTA	0.2 mg/mL PS80
F16a	Sodium adipate	6.0	10.0	None	285 mM sorbitol	6702 µM methionine	0.2 mg/mL PS80
F16	Sodium adipate	6.0	10.0	None	265 mM sorbitol	6702 µM methionine	0.2 mg/mL PS80

PS 80 = polysorbate 80

[0032] Results of this experiment are provided in FIG. 1. The inventors found that the chelators EDTA and DTPA, as well as the oxidation-scavenger methionine are effective in preventing antibody oxidation, as indicated by low oxidation rates. Furthermore, methionine, followed by DTPA, proved especially useful in protecting the antibody from oxidation in the concentration ranges used. Moreover, methionine countered oxidation strongest with diprotic acids as buffers, such as succinic acid or adipic acid.

Example 2: Analysis of Formation of High Molecular Weight Species Per Month of Incubation

[0033] In a further series of experiments, formulations of table 1 above (comprising 10 mg/ml of an antibody comprising a light chain according to SEQ ID NO:8 and a heavy chain according to SEQ ID NO:9) were analyzed with respect to their impact on aggregation, i.e. formation of high

[0034] Samples were prepared and stored as in Example 1. High Molecular Weight species (HMWs) were determined using SEC-HPLC UV at sample concentrations of 1 mg/mL.

[0035] The result of this experiment is shown in FIG. 2. In particular, the data show that the antibody showed good aggregation behaviour in the tested formulations. A particularly superior aggregation behaviour was apparent when the antibody was formulated in a diprotic acid buffer (such as succinic acid, adipic acid) and/or with methionine.

Example 3: Analysis of Further Antibody

[0036] In a further series of experiments, a different antibody and different formulations were tested (light chain according to SEQ ID NO:16; heavy chain according to SEQ ID NO:17). Formulations to be analysed were selected from the following list:

TABLE 2

List of formulations, all formulations contain protein at 25 mg/mL					
Formulation ID	Buffer type	Buffer pH	Buffer conc. (mM)	Tonifier/Stabilizer	Surfactant
F1	Histidine	5.5	10	204.5 mM sucrose	0.2 mg/mL PS80
F2	Succinate	5.5	10	204.5 mM sucrose	0.2 mg/mL PS80
F3	Acetate	5.5	10	204.5 mM sucrose	0.2 mg/mL PS80
F4	Adipate	5.5	10	204.5 mM sucrose	0.2 mg/mL PS80
F5	Succinate	5.5	10	160 mM sucrose/40 mM 2-hydroxypropyl-beta-cyclodextrin	0.2 mg/mL PS80
F5a	Succinate	5.5	10	146 mM sucrose/22 mM 2-hydroxypropyl-beta-cyclodextrin	0.2 mg/mL PS80
F6	Succinate	5.5	10	204.5 mM sorbitol	0.2 mg/mL PS80

PS 80 = polysorbate 80

[0037] Compounding of samples was performed on Tecan liquid handling system. After sterile filtration, 150 μ L of each formulation (F1-F5) was placed to a storage temperature of 40° C. in 96 matrix—well type I glass tubes for up to 3 months. Aggregation was evaluated by UPLC SEC.

Degradation products were monitored by non-reduced CE-SDS and change in charge variants was evaluated by ICE. Results of experiments are provided in FIGS. 3-5 and table 3 below.

TABLE 3

SEC, nr CE - SDS and iCE data for t0, 1 month and 3 months 40° C.							
Formulation	Pull point	Description	SEC pH	% AP	nr CE-SDS % LMW	iCE % Acidic	
F1	t0	25 mg/mL protein, 10 mM His/HCl buffer, 204.5 mM Sucrose, 0.2 mg/mL PS80	5.5	0.3	1.4	27.7	
F2	t0	25 mg/mL protein, 10 mM Succinate buffer, 204.5 mM Sucrose, 0.2 mg/mL PS80	5.5	0.3	1.5	23.2	
F3	t0	25 mg/mL protein, 10 mM Acetate buffer, 204.5 mM Sucrose, 0.2 mg/mL PS80	5.4	0.4	1.5	23.1	
F4	t0	25 mg/mL protein, 10 mM Adipate buffer, 204.5 mM Sucrose, 0.2 mg/mL PS80	5.5	0.3	1.5	23.6	
F5	t0	25 mg/mL protein, 10 mM Acetate buffer, 160 mM Sucrose/40 mM 2 hydroxypropyl-beta-cyclodextrin, 0.2 mg/mL PS80	5.5	0.2	1.5	24.8	
F1	1 Mo 40° C.	25 mg/mL protein, 10 mM His/HCl buffer, 204.5 mM Sucrose, 0.2 mg/mL PS80	5.5	0.7	4.7	41.9	
F2	1 Mo 40° C.	25 mg/mL protein, 10 mM Succinate buffer, 204.5 mM Sucrose, 0.2 mg/mL PS80	5.5	1.0	4.1	46.1	
F3	1 Mo 40° C.	25 mg/mL protein, 10 mM Acetate buffer, 204.5 mM Sucrose, 0.2 mg/mL PS80	5.4	0.7	4.3	44.4	
F4	1 Mo 40° C.	25 mg/mL protein, 10 mM Adipate buffer, 204.5 mM Sucrose, 0.2 mg/mL PS80	5.5	0.9	4.0	44.9	
F5	1 Mo 40° C.	25 mg/mL protein, 10 mM Acetate buffer, 160 mM Sucrose/40 mM 2 hydroxypropyl-beta-cyclodextrin, 0.2 mg/mL PS80	5.5	0.6	4.3	46.5	
F1	3 Mo 40° C.	25 mg/mL protein, 10 mM His/HCl buffer, 204.5 mM Sucrose, 0.2 mg/mL PS80	5.5	3.8	11.3	73.4	
F2	3 Mo 40° C.	25 mg/mL protein, 10 mM Succinate buffer, 204.5 mM Sucrose, 0.2 mg/mL PS80	5.5	3.4	9.0	67.4	
F3	3 Mo 40° C.	25 mg/mL protein, 10 mM Acetate buffer, 204.5 mM Sucrose, 0.2 mg/mL PS80	5.4	2.6	8.8	65.0	
F4	3 Mo 40° C.	25 mg/mL protein, 10 mM Adipate buffer, 204.5 mM Sucrose, 0.2 mg/mL PS80	5.5	3.4	9.3	64.8	
F5	3 Mo 40° C.	25 mg/mL protein, 10 mM Acetate buffer, 160 mM Sucrose/40 mM 2 hydroxypropyl-beta-cyclodextrin, 0.2 mg/mL PS80	5.5	2.1	9.9	66.7	

SEQUENCE LISTING

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

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Asn

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 35 40 45
 Arg Leu Leu Ile Tyr Leu Ala Ser Tyr Leu Glu Ser Gly Val Pro Ala
 50 55 60
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
 65 70 75 80
 Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Ser Arg
 85 90 95
 Asp Leu Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
 100 105 110
 Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 115 120 125
 Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 130 135 140
 Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
 145 150 155 160
 Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
 165 170 175
 Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
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 Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45
 Gly Gly Ile Asn Pro Ser Asn Gly Gly Thr Asn Phe Asn Glu Lys Phe
 50 55 60
 Lys Asn Arg Val Thr Leu Thr Thr Asp Ser Ser Thr Thr Thr Ala Tyr
 65 70 75 80
 Met Glu Leu Lys Ser Leu Gln Phe Asp Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Arg Asp Tyr Arg Phe Asp Met Gly Phe Asp Tyr Trp Gly Gln
 100 105 110
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
 115 120 125

-continued

Phe	Pro	Leu	Ala	Pro	Cys	Ser	Arg	Ser	Thr	Ser	Glu	Ser	Thr	Ala	Ala
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Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser
145					150					155					160
Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val
				165					170					175	
Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro
			180					185					190		
Ser	Ser	Ser	Leu	Gly	Thr	Lys	Thr	Tyr	Thr	Cys	Asn	Val	Asp	His	Lys
		195					200					205			
Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Ser	Lys	Tyr	Gly	Pro
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Pro	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Phe	Leu	Gly	Gly	Pro	Ser	Val
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Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr
				245					250					255	
Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	Gln	Glu	Asp	Pro	Glu
			260					265					270		
Val	Gln	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys
		275					280					285			
Thr	Lys	Pro	Arg	Glu	Glu	Gln	Phe	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser
	290					295					300				
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Cys	Lys	Val	Ser	Asn	Lys	Gly	Leu	Pro	Ser	Ser	Ile	Glu	Lys	Thr	Ile
				325					330					335	
Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro
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Pro	Ser	Gln	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu
		355					360					365			
Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn
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Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser
385					390					395					400
Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Arg	Leu	Thr	Val	Asp	Lys	Ser	Arg
				405					410					415	
Trp	Gln	Glu	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu
			420					425					430		
His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Leu	Gly	Lys	
		435					440						445		

1. A stable pharmaceutical formulation comprising an IgG4 antibody, a buffer, a surfactant, a stabilizer selected from the group consisting of sugars and sugar alcohols, and further optionally a chelator and/or oxygen-scavenger.

2. The stable formulation according to claim 1, wherein the formulation is a liquid formulation and wherein the formulation comprises sorbitol and/or sucrose.

3. The stable formulation according to claim 1, wherein the buffer is an acetate buffer, adipate buffer, histidine buffer, citrate buffer or succinate buffer.

4. The stable formulation according to claim 1, wherein the buffer has a pH of 5 to 6.

5. The stable formulation according to claim 1, wherein the surfactant is selected from polysorbate 20, polysorbate 80, a poloxamer and combinations thereof.

6. The stable formulation according to claim 1, wherein the formulation comprises a chelator and/or oxygen-scavenger and the chelator/oxygen-scavenger is selected from the group consisting of diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA), methionine and combinations thereof.

7. The stable, formulation according to claim 1, wherein the composition comprises a salt.

8. The stable formulation according to claim 1, wherein the composition comprises antibody in a concentration in the range of about 5 to 50 mg/ml.

9. The stable formulation according to claim 1, wherein the formulation comprises:

- a) a succinate buffer, EDTA, sorbitol and polysorbate 80,
- b) a histidine buffer, sodium chloride, EDTA, sorbitol and polysorbate 20,
- c) a sodium adipate buffer, methionine, sorbitol and polysorbate 80,
- d) a succinate buffer, sucrose and polysorbate 80,
- e) an acetate buffer, sucrose and polysorbate 80, or
- f) an adipate buffer, sucrose and polysorbate 80.

10. The stable formulation according to claim 1, wherein the formulation comprises:

- a) a 20 mM sodium succinate buffer, pH 6.0, 265 mM sorbitol, 20 μ M or 50 μ M EDTA, and 0.2 mg/ml polysorbate 80, and no sodium chloride and/or sodium sulphate,
- b) a 10 mM histidine/HCl buffer, pH 6.0, 50 mM sodium chloride, 215 mM sorbitol, 20 μ M or 50 μ M EDTA, and 0.2 mg/ml polysorbate 20,
- c) a 10 mM histidine/HCl buffer, pH 6.0, 50 mM sodium chloride, 165 mM sorbitol, 20 μ M or 50 μ M EDTA, and 0.2 mg/ml polysorbate 20,

d) a 10 mM sodium adipate buffer, pH 6.0, 265 mM or 285 mM sorbitol, 6702 μ M methionine and 0.2 mg/ml polysorbate 80,

- e) a 10 mM adipate buffer, pH 5.5, 204.5 mM sucrose, and 0.2 mg/ml polysorbate 80 and optionally a chelator and/or oxygen-scavenger,
- f) a 10 mM acetate buffer, pH 5.5, 204.5 mM sucrose, and 0.2 mg/ml polysorbate 80, and optionally a chelator and/or oxygen-scavenger, or
- g) a 10 mM succinate buffer, pH 5.5, 204.5 mM sucrose, and 0.2 mg/ml polysorbate 80, and optionally a chelator and/or oxygen-scavenger.

11. The stable formulation according to claim 9, wherein the formulation comprises 2 hydroxypropyl-beta-cyclodextrin.

12. An article of manufacture comprising a container, a stable pharmaceutical formulation of claim 1, and instructions for its use.

13. The article of claim 12, wherein the article is a pre-filled syringe or glass vial.

14. (canceled)

15. A method of treating a disease of the human or animal body comprising administering the stable liquid pharmaceutical formulation of claim 1 to a patient.

* * * * *