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(54) Title: AQUEOUS PHARMACEUTICAL FORMULATIONS

(57) Abstract: The present invention belong to the field of biotechnology, in particular to the field of galenics. The present invention is directed to an aqueous pharmaceutical composition comprising 1-50 mg/ml of a human or humanized full length IgG_4, e.g. natalizumab, and a formulation selected from (i) to (iii), as further defined in the claims. The present invention is further directed to a method for producing said pharmaceutical composition, and medical uses thereof. Finally, also provided is a method for stabilizing human or humanized full length IgG_4, e.g. natalizumab, as further defined in the claims.

Aqueous Pharmaceutical Formulations

The present invention is directed to an aqueous pharmaceutical composition comprising 1-50 mg/ml of a human or humanized full length IgG_4, e.g. natalizumab, and a formulation selected from (i) to (iii), as further defined in the claims. The present invention is further directed to a method for producing said pharmaceutical composition, and medical uses thereof. Finally, also provided is a method for stabilizing human or humanized full length IgG_4, e.g. natalizumab, as further defined in the claims.

BACKGROUND OF THE INVENTION

There are four different subclasses of IgG antibodies, IgG_1 to IgG_4, which differ in their sequence and function. For example, IgG_3 is characterized by a longer and more flexible hinge domain and the presence of 11 inter-heavy chain disulfide bridges, while IgG_1 and IgG_4 comprises only 2, and IgG_2 comprises 4 inter-heavy chain disulfide bridges. Apart thereof, IgG_4 antibodies are secreted as both, disulfide-linked tetramers and half-molecules, linked by strong non-covalent interactions, which is not observed in other IgG subclasses. Moreover, IgG_4 can undergo Fab-arm exchange. The particularities of IgG_4 structure and function are reviewed, for example, in Davies & Sutton, Immunol. Rev. 268(1): 139-159 (2015). As a consequence, requirements for a stable aqueous pharmaceutical composition for human or humanized IgG_4 antibodies differ from those reported for other human IgG subclasses such as IgG_1, IgG_2 and IgG_3.

The recombinant therapeutic monoclonal antibody natalizumab is an IgG_4 full-length antibody humanized from a murine monoclonal antibody that binds to the $\alpha_4\beta_1$ integrin (also known as VLA-4 or CD49d-CD29) and $\alpha_4\beta_7$ integrin, and blocks the interaction of said α_4 integrins with their respective receptors VCAM-1 and MadCAM-1 which are expressed on endothelial cells. See also WO 95/19790. α_4-integrin is required for inflammatory lymphocytes to attach to and pass through the cell layers lining the intestine and blood-brain-barrier.

Natalizumab is marketed by Biogen MA Inc. under the name Tysabri, and was previously named Antegren. It has FDA-approval for the treatment of multiple sclerosis and Crohn's disease, and EMEA approval for the treatment of multiple sclerosis. Recently, it was suggested that natalizumab could also be used in a combination treatment of B-cell malignancies, where it is intended to overcome the resistance to rituximab. Natalizumab is typically administered by intravenous infusion. According to the Scientific Discussion available from the EMEA, natalizumab is recombinantly produced in a NS/0 murine myeloma cell line. The antibody is then purified using Protein A affinity chromatography and hydrophobic interaction chromatography, followed by a buffer exchange and concentration by ultrafiltration/diafiltration.

In the current commercialized formulation, natalizumab is simply formulated in a PBS-Tween standard buffer comprising 10 mM sodium phosphate, 140 mM sodium chloride, 0.02% (w/v) polysorbate 80, pH 6.1, which does not comprise a polyol. See also WO 2004/071439 A1, EP 2 236 154 A1, and the preferred embodiments therein (e.g. claim 34 of WO 2004/071439 A1). WO 2004/071439 A1 further discloses a solution for lyophilisation of 20 mg/ml natalizumab comprising 5 mM histidine, 20 mg/ml sucrose, 0.02% polysorbate 80 at pH 6.0 (cf. claim 40).

WO 2014/160490 discloses antibody formulations comprising 15-100 mg/ml monoclonal antibody, 15-35 mM phosphate buffer, pH 5.5-7.0, 0.01% - 0.1% surfactant and trehalose in an amount of up to 2.7% (w/v).

Spiess et al. Journal of Biological Chemistry 288(37): 26583-26593 (2013) disclose IgG_4 antibody formulations comprising 45 mg/ml antibody, 0.2 M arginine succinate, pH 5.5, 0.02% polysorbate 20.

EP 1 314 437 A1 discloses formulations of humanized IgG_1 antibody hPM-1. While EP 1 314 437 A1 teaches the use of formulations comprising 1-50 mM histidine at a pH of 5.5-6.2, preferred embodiments comprise 5-10 mM histidine at a pH of 6.2-7.5. According to paragraph [0062] of EP 1 314 437 A1, addition of sodium chloride resulted in increased aggregation, for which reason it is taught that these formulations shall not contain sodium chloride (cf. claim 8 of EP 1 314 437 A1).

WO 98/56418 A1 discloses pharmaceutical compositions of the humanized IgG_1 antibodies H52 (directed against CD18) and C2B8 (directed against CD20). While initially, histidine 10 mM histidine buffer buffer, 140 mM sodium chloride, 0.01% Tween-20, pH 6.0 is tested, the authors come to the conclusion that it is more desirable to use a sugar as the tonicifier, rather than a salt, so as to minimize oxidation at low pH (cf. page 27, lines 8-9). The finally preferred formulation is set out in the claims of WO 98/56418 A1, and comprises histidine or acetate buffer in combination with a polyol, and polysorbate, pH 4.5-6.0. The most preferred embodiment of claim 21 comprises 20-30 mM acetate buffer at about pH 5, 1-15% (w/v) trehalose, 0.01-0.03% polysorbate, and 0.5 to 1% benzyl alcohol.

US 8,420,081 and US 8,883,146 describe pharmaceutical compositions for human IgG_1 1D4.7 and 13C5.5 having a conductivity of less than 2.5 mS/cm. The pharmaceutical compositions of the present disclosure are neither described nor suggested.

It is thus the object of the present invention to provide pharmaceutical compositions of human or humanized IgG_4. Another object of the present invention is to provide pharmaceutical compositions for human or humanized IgG_4 which can be used as alternatives to formulations known from the prior art.

The present invention demonstrates that the stability can be maintained by formulating human or humanized IgG_4 in the pharmaceutical compositions of the present disclosure. The pharmaceutical compositions of the present invention protect human or humanized

IgG_4 against degradation induced by temperature stress or mechanical stress. The increased physical stability of the pharmaceutical composition results in a long shelf-life and ensures product safety.

SUMMARY OF THE INVENTION

The present inventors have conducted a research programme and found aqueous pharmaceutical compositions for a human or humanized full length IgG_4 antibody, which exhibits advantageous properties, e.g. a good stability and thereby a long shelf-life. In case of natalizumab, the formulations show a stability comparable to the commercial formulation. In particular, it is provided an aqueous pharmaceutical composition comprising 1-50 mg/ml of a human or humanized full length IgG_4, and a formulation selected from

(i) a formulation comprising 10-25 mM histidine buffer, 130-180 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5; or

(ii) a formulation comprising 25-30 mM phosphate buffer, 5-10 % (w/v) of a polyol, and 0.001-0.5 % (w/v) of a surfactant, pH 5.0-6.9; or

(iii) a formulation comprising 10-25 mM histidine buffer, 10-50 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5.

In a preferred embodiment, the human or humanized full-length IgG_4 is natalizumab.

Further provided is an article of manufacture, comprising a pharmaceutical composition as described herein and instructions for use of said composition, preferably wherein said pharmaceutical composition is contained in a pre-filled vial or in a pre-filled syringe, more preferably wherein said pharmaceutical composition is contained in a pre-filled vial.

In addition, a method for producing an aqueous pharmaceutical composition as described herein is provided, the method comprising combining 1-50 mg/ml of a human or humanized full-length IgG_4 with a formulation selected from

(i) a formulation comprising 10-25 mM histidine buffer, 130-180 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5; or

(ii) a formulation comprising 25-30 mM phosphate buffer, 5-10 % (w/v) of a polyol, and 0.001-0.5 % (w/v) of a surfactant, pH 5.0-6.9; or

(iii) a formulation comprising 10-25 mM histidine buffer, 10-50 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5.

Also provided is a method for stabilizing a human or humanized full length IgG_4, comprising combining 1-50 mg/ml of a human or humanized full length IgG_4 with a formulation selected from

(i) a formulation comprising 10-25 mM histidine buffer, 130-180 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5; or

(ii) a formulation comprising 25-30 mM phosphate buffer, 5-10 % (w/v) of a polyol, and 0.001-0.5 % (w/v) of a surfactant, pH 5.0-6.9; or

(iii) a formulation comprising 10-25 mM histidine buffer, 10-50 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5,

thereby preparing a stable aqueous pharmaceutical composition.

The stable aqueous pharmaceutical composition described herein is particularly useful in the treatment of multiple sclerosis or Crohn's disease.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

More specifically, the present disclosure provides an aqueous pharmaceutical composition comprising, preferably consisting of, 1-50 mg/ml of a human or humanized full length IgG_4, and a formulation selected from

(i) a formulation comprising 10-25 mM histidine buffer, 130-180 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5; or

(ii) a formulation comprising 25-30 mM phosphate buffer, 5-10 % (w/v) of a polyol, and 0.001-0.5 % (w/v) of a surfactant, pH 5.0-6.9; or

(iii) a formulation comprising 10-25 mM histidine buffer, 10-50 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5.

Among the four formulations (i)-(iii), formulation (i) is particularly preferred.

The aqueous pharmaceutical composition described herein is not intended for lyophilisation, and has not been previously lyophilized. In other words, the pharmaceutical composition described herein is intended to be stored in "dissolved" form, as also apparent from the indicated concentration ranges and the term "aqueous". In a preferred embodiment, the pharmaceutical composition is in liquid form.

The aqueous pharmaceutical composition described herein are expected to be stable. The term "stable" as used herein is intended to mean that the composition retains its physical stability, and/or conformational stability and/or colloidal stability upon storage, as further described below. The composition may also retain chemical stability and/or biological activity, as further described below.

The term "physical stability" refers to the ability of a product to maintain its physical dimensions when exposed to conditions normally encountered in its service environment. In particular, the pharmaceutical composition described herein exhibits a comparable physical stability to the same human or humanized IgG_4 antibody formulated at the same concentration in 10 mM sodium phosphate, 140 mM sodium chloride, 0.02% (w/v) polysorbate 80, pH 6.1. Physical stability can be measured in terms of precipitation, degradation, and/or denaturation, and can be measured, for example, by visual inspection

of color and/or clarity, size exclusion chromatography, or micro flow imaging, the determination and assessment of which is further described in the examples below.

In another preferred embodiment, the pharmaceutical composition exhibits less sub-visible particles (>10µm) as compared to the same human or humanized IgG_4 antibody formulated at the same concentration in 10 mM sodium phosphate, 140 mM sodium chloride, 0.02% (w/v) polysorbate 80, pH 6.1 after three cycles of freezing from room temperature to -80°C for 8h and then thawing for 2h at room temperature. In a more preferred embodiment, the pharmaceutical composition exhibits less than 99% sub-visible particles, preferably less than 95%, more preferably less than 90%, more preferably less than 85%, more preferably less than 80%, more preferably less than 75%, even more preferably less than 70%, and most preferably less than 65% sub-visible particles (>10µm) as determined by microflow imaging after three cycles of freezing from room temperature to -80°C for 8h and then thawing for 2h at room temperature. Microflow imaging is a technique that allows to characterize sub-visible particles in the range of 2µm to 200µm in a given formulation. Before sample preparation, the system is primed with related formulation buffer to optimize illumination. Samples are then prepared by gently mixing in circular motions after removal from storage conditions. Then the vials are opened and 1 ml of sample is withdrawn and loaded in the inlet port of the microflow imaging system. Data is acquired using the following parameters: Sample dispensed: 0.9 ml; purge volume: 0.2 ml; sample analyzed volume: 0.59 ml; edge particles rejection: enabled; fill particles: enabled; and the data is analyzed by setting filters above 10µm. Preferably the samples are neat at target concentration of about 20 mg/ml. Further guidance is provided in the examples below.

In another preferred embodiment, the pharmaceutical composition exhibits less than 1% aggregation products, in particular less than 0.95%, such as less than 0.9%, or even less than 0.85%, as determined after four weeks of storage at 40°C by size exclusion chromatography.

In addition or alternatively, the pharmaceutical composition exhibits an increase of less than 0.5% of aggregation products, in particular less than 0.45%, such as less than 0.4%, or even less than 0.35%, as determined after four weeks of storage at 40°C by size exclusion chromatography. Certain formulations of the present disclosure even exhibit less than 0.5%, in particular less than 0.4% such as less than 0.35% aggregation products as determined after four weeks of storage at 40°C by size exclusion chromatography.

In addition, the pharmaceutical composition exhibits less aggregation products as compared to the same human or humanized IgG_4 antibody formulated at the same concentration in 10 mM sodium phosphate, 140 mM sodium chloride, 0.02% (w/v) polysorbate 80, pH 6.1, as determined after at four weeks of storage at 40°C, 25°C, or

5°C, preferably at 40°C, by size exclusion chromatography. In a more preferred embodiment, the pharmaceutical composition exhibits less than 99% aggregation products, preferably less than 98.5%, more preferably less than 98%, even more preferably less than 97.5% aggregation products, and most preferably less than 97% aggregation products as determined after four weeks of storage at 40°C, 25°C, or 5°C, preferably at 40°C, by size exclusion chromatography.

In addition, or alternatively, the pharmaceutical composition may exhibit less degradation products as compared to the same human or humanized IgG4 antibody formulated at the same concentration in 10 mM sodium phosphate, 140 mM sodium chloride, 0.02% (w/v) polysorbate 80, pH 6.1 as determined after storage for 12, preferably 20, more preferably 24, more preferably 48, more preferably 72, and most preferably 96 weeks at 40°C by size exclusion chromatography. In a more preferred embodiment, the pharmaceutical composition may exhibit less than 99% degradation products, preferably less than 98.5%, more preferably less than 98%, even more preferably less than 97.5% degradation products, and most preferably less than 97% degradation products as determined after storage for 12, preferably 20, more preferably 24, more preferably 48, more preferably 72, and most preferably 96 weeks at 40°C by size exclusion chromatography. Size exclusion chromatography is performed using UPLC system equipped with Waters BEH200 SEC 1.7 um 4.6x150mm at column temperature 30 ± 0.5 °C. The mobile phase is 100 mM sodium phosphate buffer with 200 mM sodium chloride, pH 6.8 ± 0.05 at a flow rate of 0.3 ml/min. The samples are diluted to 1 mg/ml in the respective buffer and then a volume of 2 µl is injected to the system. Detection is carried out at 210 nm.

Preferably, the pharmaceutical composition exhibits less than 3% of IgG_4 half antibodies, in particular less than 2.9%, preferably less than 2.5%, more preferably less than 2.2%, and most preferably less than 2.1%, after storage for four weeks at 40°C, as determined by polyacrylamide gel electrophoresis under non-reducing conditions and colloidal Coomassie Blue gel staining. Polyacrylamide gel electrophoresis under non-reducing conditions and colloidal Coomassie Blue gel staining may be carried out using the Experion® electrophoresis system (Biorad) as described in Example 3 below.

In another preferred embodiment, the pharmaceutical composition exhibits less percent half antibodies as compared to the same human or humanized IgG_4 antibody formulated at the same concentration in 10 mM sodium phosphate, 140 mM sodium chloride, 0.02% (w/v) polysorbate 80, pH 6.1, after storage for four weeks at 40°C, as determined by polyacrylamide gel electrophoresis under non-reducing conditions and colloidal Coomassie Blue gel staining. Polyacrylamide gel electrophoresis under non-reducing conditions and colloidal Coomassie Blue gel staining may be carried out using the Experion® electrophoresis system (Biorad) as described in Example 3 below.

The term “conformational stability” refers to the denaturation temperature and denaturation enthalpy of proteins and other macromolecules in solution as a measure of molecular stability in a given environment. Conformational stability can be measured in, for example, by Differential Scanning Calorimetry (DSC). DSC measures the amount of heat that is absorbed or released from biomolecules in solution during heating. Native proteins respond to heating by unfolding (thermal denaturation) at a characteristic temperature (Tm). The more intrinsically stable the biopolymer is the higher is the midpoint temperature of the unfolding transition (Tm) and the transition enthalpy. Measurements are carried out in scanning mode in a temperature range of 20°C to 100°C with heating and scanning rate of 1°C/min; a buffer scan is conducted prior to each sample run to generate a baseline. Samples are prepared via dialysis against the corresponding formulation buffer and diluted to about 1 mg/ml. The corresponding dialysis buffer is used as buffer scan. The exact sample concentration after dialysis is determined by UV (280 nm) absorption.

In a preferred embodiment, the human or humanized IgG_4 have their T_{m1} (CH_2 domain unfolding) in the range from 65°C to 70°C, as determined by DSC. In another preferred embodiment, the pharmaceutical composition described herein exhibits a higher conformational stability as compared to the same human or humanized IgG_4 antibody formulated at the same concentration in 10 mM sodium phosphate, 140 mM sodium chloride, 0.02% (w/v) polysorbate 80, pH 6.1. Even more preferably, the Tm is more than 0.1 °C higher in the pharmaceutical composition described herein as compared to the same human or humanized IgG_4 antibody formulated at the same concentration in 10 mM sodium phosphate, 140 mM sodium chloride, 0.02% (w/v) polysorbate 80, pH 6.1; more preferably the T_m is more than 0.25°C, more preferably more than 0.5 °C, more preferably more than 0.75°C, more preferably more than 1.0 °C, even more preferably more than 1.25 °C, still more preferably more than 1.5 °C, and most preferably more than 1.75°C higher; in particular after three cycles of freezing from room temperature to -80°C for 8h and then thawing for 2h at room temperature.

The term “colloidal stability” refers to the molecules’ interaction in Newtonian solutions, as determinable by Composition gradient multi-angle light scattering (CG-MALS). An interaction between protein molecules in solution is characterized by changes in their light scattering behavior at different concentrations via CG-MALS. The characteristic parameter to measure molecule interactions is the second virial coefficient A_2. A_2 is characteristic for a macromolecule and its solvent, and describes molecular interactions between the dissolved macromolecules. A negative A_2 indicates attractive interactions whereas a positive A_2 is characteristic for repulsive interactions between the dissolved molecules.

The CG-MALS technique is based on a concentration series of a macromolecular solution which is directly injected into the flow cell of a multi angle light scattering detector. After each injection the flow is stopped to permit the reaction to reach equilibrium. The apparent molecular weight (Mw_{app}) is determined for each step in the gradient by analyzing light scattering and concentration data by the following equation:

$$Mw_{app} \models \frac{R(\theta,c)}{K \times c}$$

wherein

Mw_{app}: apparent molecular weight

R(θ,c) **:** excess Rayleigh ratio of the solution as a function of scattering angle θ and concentration c. It is directly proportional to the intensity of the excess light scattered by the solute and the light scattered by the pure solvent.

K: Optical constant ($4p^2(dn/dc)^2n_0^2/N_al_0^4$)

c: Concentration

Significant interactions between macromolecules manifest as changes in Mw_{app} vs. concentration.

A2 calculation is then conducted via Zimm plot analysis according to:

$$\frac{K \cdot c}{R(\theta,c)} = \frac{1}{M_w P(\theta)} + A_2 c$$

wherein

R(θ,c) **:** excess Rayleigh ratio of the solution as a function of scattering angle θ and concentration c. It is directly proportional to the intensity of the excess light scattered by the solute and the light scattered by the pure solvent.

Mw: Weight average molecular weight

A_2: 2nd virial coefficient

c: Concentration

K: Optical constant ($4p^2(dn/dc)^2n_0^2/N_al_0^4$)

P(θ): describes the angular dependence of the scattered light, and can be related to the rms

radius. Below rms radii of 30 nm this term becomes 0.

In a preferred embodiment, the pharmaceutical composition described herein exhibits a higher colloidal stability as compared to the same human or humanized IgG_4 antibody formulated at the same concentration in 10 mM sodium phosphate, 140 mM sodium chloride, 0.02% (w/v) polysorbate 80, pH 6.1, as determined by the calculation of A_2. Even more preferably, the A_2 of the pharmaceutical composition is higher than -0.125, more

preferably higher than -0.120, more preferably higher than -0.115, even more preferably higher than -0.110, and most preferably higher than -0.105.

The term “chemical stability” as used herein refers to chemical alterations of the human or humanized IgG_4. Chemical alterations include charge alteration (e.g. occurring as results of deamidation) and oxidation of certain amino acids in the sequence, but also includes degradation of the protein at several independent sites. Such modifications may result in the presence of many different species in the final product. Monoclonal antibodies therefore display considerable heterogeneity that can be characterized by ion exchange liquid chromatography (IEX-LC). In a preferred embodiment, the separation is carried out by Cation Exchange Ultra Performance Liquid Chromatography on UPLC H-Class Bio System using UV detection under Empower™ Software control. The Waters Protein-Pak Hi Res SP (7 µm, 4.6 mm i.d. × 100 mm) is used for testing applying a linear gradient of NaCl. Eluents were: buffer A (14mM NaPi buffer pH 6.0) and buffer B (10.5mM NaPi buffer pH 6.0, 0.125 M NaCl). Gradient starts with pre-equilibration of 100% buffer A in 2 min. Elution gradient starts from 10% to 30% of buffer B in 25 min, followed by a second elution step for 5 min at 30% to 60% B and re-equilibration with 90% solvent A. The total run time is 40 min. The flow rate is 0.7 ml/min. The column temperature is 40°C and elution is monitored at 220 nm. For data evaluation Waters Empower 3 software is used. The peak assignment is performed by retention time. The sample composition is determined by detecting peaks based on their retention time and the relative proportions of each peak are calculated from the peak areas. The final results are presented as a sum of acidic species, main peak and sum of basic species. Exemplary results of charge variants content for a reference product (range based on 20 batches testing) are presented in the following table:.

	CEX_MAIN PEAK					
Sample	**rep. No (n)**	**Average Results**	**min**	**max**	**SD**	**CV**
Referent (20 batches)	**1**	**71.3**	**69.0**	**72.7**	**0.993**	**1.4**

	CEX_SUM of ACIDIC PEAKS					
Sample	**rep. No (n)**	**Average Results**	**min**	**max**	**SD**	**CV**
Referent (20 batches)	**1**	**13.0**	**10.3**	**15.3**	**1.393**	**10.7**

	CEX_SUM of BASIC PEAKS					
Sample	**rep. No (n)**	**Average Results**	**min**	**max**	**SD**	**CV**
Referent (20 batches)	**1**	**15.7**	**13.6**	**18.5**	**1.453**	**9.2**

The human or humanized IgG_4 may have a higher chemical stability in the pharmaceutical composition upon storage for 12, preferably 20, more preferably 24, more preferably 48,

more preferably 72, and most preferably 96 weeks at 5°C±3°C as compared to the same human or humanized IgG_4 antibody formulated at the same concentration in 10 mM sodium phosphate, 140 mM sodium chloride, 0.02% (w/v) polysorbate 80, pH 6.1, as determined by ion exchange liquid chromatography (IEX-LC). The main peak may comprise more than 73%, preferably more than 75%, more preferably more than 80%, even more preferably more than 85%, and most preferably more than 90% upon storage for 20, preferably 24, more preferably 48, more preferably 72, and most preferably 96 weeks at 5°C±3°C. In addition, or alternatively, the sum of acidic peaks may comprise less than 14.5%, preferably less than 14%, more preferably less than 13.5%, more preferably less than 13%, even more preferably less than 12.5%, and most preferably less than 12% upon storage for 12, preferably 20, more preferably 24, more preferably 48, more preferably 72, and most preferably 96 weeks at 5°C±3°C. In addition, or alternatively, the sum of basic peaks may comprise less than 12.5%, preferably less than 12%, more preferably less than 11.5%, more preferably less than 11%, even more preferably less than 10.5%, and most preferably less than 10% upon storage for 12, preferably 20, more preferably 24, more preferably 48, more preferably 72, and most preferably 96 weeks at 5°C±3°C.

The term “biological stability” means that the human or humanized IgG4 retains a higher biological activity in the pharmaceutical composition upon storage as compared to the to the same human or humanized IgG4 antibody formulated at the same concentration in 10 mM sodium phosphate, 140 mM sodium chloride, 0.02% (w/v) polysorbate 80, pH 6.1, as determined in a relevant antigen binding assay, as those below.

Mechanism of action for natalizumab involves blocking interaction of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins with their cognate receptors VCAM-1 and MadCAM-1, respectively. For example, biologic activity of natalizumab may be tested by a direct ELISA detecting binding of natalizumab to $\alpha_4\beta_1$ integrin. The principle of this method is to incubate a coated constant amount of integrin $\alpha_4\beta_1$ with serially diluted natalizumab samples. The amount of bound natalizumab is subsequently determined by a mouse, monoclonal anti-human IgG antibody, which is conjugated to horseradish peroxidase (HRP). HRP converts the chromogenic substrate TMB (3,3’,5,5’- tetramethylbenzidine) into a colored dye. The color reaction is measured spectrophotometrically at wavelength 450 nm. Data are analyzed applying 4 Parameter Logistic nonlinear regression model (4PL), which is commonly used for curve-fitting analysis in bioassays or immunoassays such as ELISAs or dose-response curves. Final result is expressed as a Relative Potency (REP) of tested sample in relation to interim reference standard established at Polpharma site. The method variability was

determined at the level of 7% coefficient variation (CV) of intermediate precision within the qualification exercise.

Alternatively, one can also measure VCAM-1 competitive binding by ELISA. The aim of this assay is to test the ability of natalizumab to inhibit interaction of $\alpha_4\beta_1$ integrin with its cognate receptor – VCAM-1 protein in a dose-dependent manner. Constant amount of the coated VCAM-1 protein is incubated with serial dilutions of natalizumab in the presence of HIS-tagged $\alpha_4\beta_1$ integrin. Solid-phase associated VCAM-1 and soluble natalizumab now compete for binding to $\alpha_4\beta_1$ integrin. The higher the natalizumab concentration the more α4β1 integrin is inhibited from binding to VCAM-1. The highest signal result is observed when no natalizumab is present. Bound HIS-tagged $\alpha_4\beta_1$ integrin is subsequently detected with a biotinylated anti-HIS-tag antibody, POD-conjugated Streptavidin and a TMB-substrate reaction at the end of the assay. Data are analyzed with 4PL fitting model. Final result is expressed as a Relative Potency (REP) of tested sample in relation to reference standard. The method variability was determined at the level of 7% coefficient variation (CV) of intermediate precision within the qualification exercise. Additionally accuracy, linearity and specificity were tested.

	VCAM-1 competitive ELISA					
Sample	**rep. No (n)**	**Average Results**	**min**	**max**	**SD**	**CV**
Referent (26 batches)	**3**	**1.044**	**0.916**	**1.200**	**0.081**	**7.74**

In still another alternative one can measure MadCAM-1 competitive binding by ELISA. The aim of this assay is to test the ability of natalizumab to inhibit interaction of $\alpha_4\beta_7$ integrin with its cognate receptor – MadCAM-1 protein in a dose-dependent manner. Constant amount of the coated $\alpha_4\beta_7$ integrin is incubated with serial dilutions of natalizumab in the presence of Fc-tagged MadCAM-1 receptor. Natalizumab and MadCAM-1 receptor now compete for binding to solid-phase associated $\alpha_4\beta_7$ integrin. The higher the natalizumab concentration the more MadCAM-1 is inhibited from binding to $\alpha_4\beta_7$ integrin. The lowest signal result is observed when no natalizumab is present. Bound natalizumab is subsequently detected with a POD-conjugated anti-human IgG antibody and a TMB-substrate reaction at the end of the assay. Data are analyzed with 4PL fitting model. Final result is expressed as a Relative Potency (REP) of tested sample in relation to reference standard. The method variability was determined at the level of 8% coefficient variation (CV) of intermediate precision within the qualification exercise. Additionally accuracy, linearity and specificity were tested.

	MadCAM-1 competitive ELISA					
Sample	rep. No (n)	Average Results	min	max	SD	CV
Referent (24 batches)	**3**	**0.995**	**0.830**	**1.170**	**0.072**	**7.27**

The biological activity of the antibody may be at least 80% of the biological activity before storage for 12, preferably 20, more preferably 24, more preferably 48, more preferably 72, and most preferably 96 weeks at 5°C±3°C (within error of the assay), more preferably at least 82%, more preferably at least 84%, more preferably at least 86%, more preferably at least 88%, more preferably at least 90%, more preferably at least 92%, more preferably at least 94%, even more preferably at least 96%, still more preferably at least 98%, and most preferably at least 99% of the original activity before storage for 12, preferably 20, more preferably 24, more preferably 48, more preferably 72, and most preferably 96 weeks at 5°C±3°C.

As exemplified below, stability of pharmaceutical compositions according to the present disclosure are assessed during a 96 weeks-stability study at intended (5 °C) as well as accelerated storage condition (25 and 40°C). In view of the results, the pharmaceutical formulation described herein is expected to be suitable for long term storage. As used herein, the term “long term storage” shall refer to storage of a composition comprising the pharmaceutical formulation for more than 4 months, preferably for more than 12, 20, 24, 48, 72 or even 96 weeks at 5°C±3°C.

The pharmaceutical composition of the present disclosure comprises 1-50 mg/ml of a human or humanized full-length IgG_4. In a preferred embodiment, said pharmaceutical composition comprises 5-50 mg/ml, preferably 10-45 mg/ml, more preferably 15-40 mg/ml, even more preferably 20-30 mg/ml of said human or humanized full-length IgG_4. In a most preferred embodiment, the pharmaceutical composition of the present disclosure comprises 20 mg/ml of said human or humanized full-length IgG_4.

The term “human or humanized full length IgG_4” as used herein means a full length immunoglobulin comprising the sequence of the constant region of the heavy chain of human IgG_4, which is well-known in the field, e.g. from Uniprot database entry P01861, and the constant region of a human light chain. The variable region of the heavy and light chain preferably also each comprise a human framework, while the CDR regions may originate from human or another species such as mouse. In a particular embodiment, the IgG_4 is a humanized IgG_4, in which the CDRs have been grafted in a human framework. Such human framework sequences of the variable domains are known in the art, and further exemplified in SEQ ID NOs 2 and 4 herein. In such humanized IgG_4 antibodies, it might be necessary that a limited number of amino acid residues (less than 10, preferably

less than 9, more preferably less than 8, more preferably less than 7, more preferably less than 6, more preferably less than 5, more preferably less than 4, more preferably less than 3, more preferably less than 2, and most preferably no more than one amino acid residue) are/is mutated in the human IgG_4 sequence, in order to improve stability and/or better integrate the grafted sequences into the human IgG_4 framework. To that end, it was recently reported that the point substitution S228P in the hinge region prevents half molecule exchange of IgG_4, and thus further stabilizes the IgG_4 molecule (Silva et al., J Biol Chem. 290(9): 5462-5469 (2015)). Examples of therapeutic humanized IgG_4 antibodies, are Natalizumab, Gemtuzumab, and Pembrolizumab.

In a still more preferred embodiment, the human or humanized full length IgG_4 is essentially identical / similar to natalizumab, most preferably, the human or humanized full length IgG_4 is natalizumab. Natalizumab is a full length humanized IgG_4 antibody with an amino acid sequence shown in amino acids 19 to 231 of SEQ ID NO: 2 and amino acids 19 to 468 of SEQ ID NO: 4. The term “essentially identical / similar to natalizumab” as used herein means that the amino acid sequence of the human or humanized IgG_4 has at least 95% identity to the amino acid sequence shown in amino acids 19 to 231 of SEQ ID NO: 2 and amino acids 19 to 468 of SEQ ID NO: 4, respectively, more preferably at least 96% identity, such as 97% identity, and most preferably 98% identity, such as 99% identity to the amino acid sequence shown in amino acids 19 to 231 of SEQ ID NO: 2 and amino acids 19 to 468 of SEQ ID NO: 4, respectively. Alternatively or additionally, the human or humanized IgG_4 may (only) differ from natalizumab by postranslational modifications, e.g. by glycosylation. Suitable procedures for changing a glycosylation pattern, such as introducing or deleting a glycosylation site, and assays for determining a glycosylation pattern are well known in the art, and described, for example, in EP 16460039, in particular on page 5 and Examples 4 and 6 therein.

The human or humanized IgG_4 may be recombinantly produced, preferably by using a mammalian cell based expression system. Preferably, said mammalian cell-based expression system is at least one selected from the group consisting of CHO cells (e.g., CHO-K1, CHO-DG44, CHO-DXB), NS/0 cells; BHK cells (e.g., BHK21); SP2/0 cells; HEK cells (e.g., HEK-293); PER-C6 cells, and/or CAP cells.

According to the Scientific Discussion available from the EMEA, natalizumab is recombinantly produced in a NS/0 murine myeloma cell line. The antibody is then purified using Protein A affinity chromatography and hydrophobic interaction chromatography, followed by a buffer exchange and concentration by ultrafiltration/diafiltration. Methods for high density cell cultures of NS/0 cells for, *inter alia*, producing natalizumab are disclosed in WO 2013/006461, which is incorporated herewith by reference in its entirety. A preferred method for producing natalizumab is disclosed in EP 16460039, in particular

Examples 1 and 2 therein, which report a method of culturing CHO DG44 cells under serum-free or protein-free culture conditions, and which express a biosimilar antibody for the monoclonal antibody natalizumab.

The pharmaceutical composition (i)-(iii) of the present disclosure comprise 0.001-0.5 % (w/v) of a surfactant. In a preferred embodiment, said formulation comprises 0.005-0.1 % (w/v), preferably 0.01-0.05 % (w/v), and more preferably 0.02 % (w/v) surfactant. Particularly suitable surfactant is a nonionic surfactant, included in the above mentioned concentration ranges. Nonionic surfactants which are pharmaceutically acceptable are known in the art and include polysorbate (Tween), poloxamer (Pluronic), polyethylene glycol alkyl ether (Brij), polyethylene glycol octylphenyl ether (Triton X-100), polypropylene glycol alkyl ether, glucoside alkyl ether, glycerol alkyl ester, and dodecyldimethylamine oxide. Among these polysorbate and poloxamer are particularly preferred, such as polysorbate 80, polysorbate 20, or poloxamer 188, included in the above mentioned concentration ranges. In a most preferred embodiment, the nonionic surfactant is polysorbate 80. Hence, in a preferred embodiment, said pharmaceutical compositions (i)-(iii) of the present disclosure comprise polysorbate 80 in a concentration of 0.005-0.1 % (w/v), preferably 0.01-0.05 % (w/v), and more preferably 0.02 % (w/v).

Formulation (i) comprises, preferably consists of, 1-50 mg/ml (preferably 5-50 mg/ml, more preferably 10-45 mg/ml, even more preferably 15-40 mg/ml, still more preferably 20-30 mg/ml, and most preferably 20 mg/ml) of a human or humanized full-length IgG_4 as further disclosed above, and 10-25 mM histidine buffer, 130-180 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant as further disclosed above, pH 5.1-6.5. In some embodiments, formulation (i) comprises 10-20 mM histidine buffer; preferably 10-15 mM histidine buffer, more preferably 10-12 mM histidine buffer, and most preferably 10 mM histidine buffer. In principle, any pharmaceutically acceptable histidine buffer may be used, and the histidine may be L-histidine, D-histidine or a mixture of D- and L-histidine. However, in a preferred embodiment the histidine is L-histidine, as this is considered to be most natural and possibly least immunogenic. It further embodiments, formulation (i) comprises 140-170 mM sodium chloride, preferably 145-165 mM sodium chloride, more preferably 150-160 mM sodium chloride, and most preferably 150 mM sodium chloride. The pH of formulation (i) is generally in the range of 5.1-6.5, while preferred embodiments are wherein in formulation (i) pH is 5.1-6.4, preferably 5.1-6.3, more preferably 5.1-6.2, more preferably 5.1-6.1, more preferably 5.2-6.0, more preferably pH is 5.3-5.9, more preferably pH is 5.4-5.8, even more preferably pH is 5.5-5.7. Most preferably formulation (i) has a pH of 5.7.

Particularly preferred formulations (i) are formulations comprising, preferably consisting of

Formulation #	L-histidine (mM)	NaCl (mM)	polysorbate 80 % (w/v)	pH
1	10	150	0.02	5.5
2	12	150	0.02	5.5
3	14	150	0.02	5.5
4	16	150	0.02	5.5
5	18	150	0.02	5.5
6	20	150	0.02	5.5
7	10	150	0.02	5.6
8	12	150	0.02	5.6
9	14	150	0.02	5.6
10	16	150	0.02	5.6
11	18	150	0.02	5.6
12	20	150	0.02	5.6
13	10	150	0.02	5.7
14	12	150	0.02	5.7
15	14	150	0.02	5.7
16	16	150	0.02	5.7
17	18	150	0.02	5.7
18	20	150	0.02	5.7
19	10	160	0.02	5.5
20	12	160	0.02	5.5
21	14	160	0.02	5.5
22	16	160	0.02	5.5
23	18	160	0.02	5.5
24	20	160	0.02	5.5
25	10	160	0.02	5.6
26	12	160	0.02	5.6

27	14	160	0.02	5.6
28	16	160	0.02	5.6
29	18	160	0.02	5.6
30	20	160	0.02	5.6
31	10	160	0.02	5.7
32	12	160	0.02	5.7
33	14	160	0.02	5.7
34	16	160	0.02	5.7
35	18	160	0.02	5.7
36	20	160	0.02	5.7
37	10	140	0.02	5.5
38	12	140	0.02	5.5
39	14	140	0.02	5.5
40	16	140	0.02	5.5
41	18	140	0.02	5.5
42	20	140	0.02	5.5
43	10	140	0.02	5.6
44	12	140	0.02	5.6
45	14	140	0.02	5.6
46	16	140	0.02	5.6
47	18	140	0.02	5.6
48	20	140	0.02	5.6
49	10	140	0.02	5.7
50	12	140	0.02	5.7
51	14	140	0.02	5.7
52	16	140	0.02	5.7
53	18	140	0.02	5.7

54	20	140	0.02	5.7

In some embodiments, formulation (i) further comprises 5-30 mM methionine, preferably 6-25 mM methionine, more preferably 7-20 mM methionine, more preferably 8-15 mM methionine, more preferably 9-12 mM methionine, and most preferably 10 mM methionine. In principle, any pharmaceutically acceptable methionine may be used, and the methionine may be L- methionine, D- methionine or a mixture of D- and L- methionine. However, in a preferred embodiment the methionine is L- methionine, as this is considered to be most natural and possibly least immunogenic.

In addition to methionine, formulation (i) may additionally comprises 5-50 mM glycine, preferably 10-45 mM glycine, more preferably 15-40 mM glycine, more preferably 20-35 mM glycine, more preferably 25-30 mM glycine, and most preferably 30 mM glycine.

As shown in the examples, in certain embodiments it can be advantageous to add very low amounts of a phosphate buffer in addition to the histidine buffer. Hence, in some embodiments, formulation (i) further comprises 1-5 mM of a phosphate buffer, preferably 2-4 mM of a phosphate buffer, more preferably 2.5-3 mM of a phosphate buffer, and most preferably 2.5 mM of a phosphate buffer. While in principle any pharmaceutically acceptable phosphate buffer may be used, it is preferred that the phosphate buffer of formulation (i) of the present disclosure is sodium phosphate buffer, e.g., in order to avoid administration of potassium ions which can affect heart function.

Very preferred embodiments of formulation (i) of the present disclosure consists of

- 10 mM L-histidine buffer, 150 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.
- 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 150 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.
- 25 mM L-histidine buffer, 150 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.
- 10 mM L-histidine buffer, 150 mM NaCl, 10mM L-methionine, and 0.02 % (w/v) polysorbate 80, pH 5.7.
- 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 150 mM NaCl, 10mM L-methionine, and 0.02 % (w/v) polysorbate 80, pH 5.7.
- 25 mM L-histidine buffer, 150 mM NaCl, 10mM L-methionine, and 0.02 % (w/v) polysorbate 80, pH 5.7.
- 10 mM L-histidine buffer, 150 mM NaCl, 10mM L-methionine, 30 mM glycine, and 0.02 % (w/v) polysorbate 80, pH 6.1.
- 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 150 mM NaCl, 10mM L-methionine, 30 mM glycine, and 0.02 % (w/v) polysorbate 80, pH 6.1.
- 25 mM L-histidine buffer, 150 mM NaCl, 10mM L-methionine, 30 mM glycine, and 0.02 % (w/v) polysorbate 80, pH 6.1.
- 10 mM L-histidine buffer, 150 mM NaCl, and 0.2 % (w/v) polysorbate 80, pH 5.7.

In a most preferred embodiment, said formulation (i) consists of 10 mM L-histidine buffer, 150 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.

Formulation (ii) comprises, preferably consists of, 1-50 mg/ml (preferably 5-50 mg/ml, more preferably 10-45 mg/ml, even more preferably 15-40 mg/ml, still more preferably 20-30 mg/ml, and most preferably 20 mg/ml) of a human or humanized full-length IgG_4 as further disclosed above, and 25-30 mM phosphate buffer, 5-10 % (w/v) of a polyol (preferably 5% (w/v)), and 0.001-0.5 % (w/v) of a surfactant as further disclosed above, pH 5.0-6.9. While in principle any pharmaceutically acceptable phosphate buffer may be used, it is preferred that the phosphate buffer of the formulation (ii) of the present disclosure is sodium phosphate buffer, e.g., in order to avoid administration of potassium ions which can affect heart function. Likewise, in principle any pharmaceutically acceptable polyol may be used, but it is preferred that the polyol in formulation (ii) is selected from sorbitol, mannitol, xylitol, and inositol, among which sorbitol is most preferred. It is further preferred that the pH of formulation (i) is 5.5-6.8, preferably 5.8-6.7, more preferably 6.0-6.5, and most preferably a pH of 6.1-6.3.

Particularly preferred formulations (ii) are

Formulation #	$NaPO_4$ (mM)	sorbitol % (w/v)	polysorbate 80 % (w/v)	pH
55	25	5	0.02	6.0
56	26	5	0.02	6.0
57	27	5	0.02	6.0
58	28	5	0.02	6.0
59	29	5	0.02	6.0
60	30	5	0.02	6.0
61	25	5	0.02	6.1
62	26	5	0.02	6.1
63	27	5	0.02	6.1
64	28	5	0.02	6.1
65	29	5	0.02	6.1
66	30	5	0.02	6.1
67	25	5	0.02	6.2
68	26	5	0.02	6.2

69	27	5	0.02	6.2
70	28	5	0.02	6.2
71	29	5	0.02	6.2
72	30	5	0.02	6.2
73	25	5	0.02	6.3
74	26	5	0.02	6.3
75	27	5	0.02	6.3
76	28	5	0.02	6.3
77	29	5	0.02	6.3
78	30	5	0.02	6.3
79	25	7	0.02	6.0
80	26	7	0.02	6.0
81	27	7	0.02	6.0
82	28	7	0.02	6.0
83	29	7	0.02	6.0
84	30	7	0.02	6.0
85	25	7	0.02	6.1
86	26	7	0.02	6.1
87	27	7	0.02	6.1
88	28	7	0.02	6.1
89	29	7	0.02	6.1
90	30	7	0.02	6.1
91	25	7	0.02	6.2
92	26	7	0.02	6.2
93	27	7	0.02	6.2
94	28	7	0.02	6.2
95	29	7	0.02	6.2

96	30	7	0.02	6.2
97	25	7	0.02	6.3
98	26	7	0.02	6.3
99	27	7	0.02	6.3
100	28	7	0.02	6.3
101	29	7	0.02	6.3
102	30	7	0.02	6.3
103	25	10	0.02	6.0
104	26	10	0.02	6.0
105	27	10	0.02	6.0
106	28	10	0.02	6.0
107	29	10	0.02	6.0
108	30	10	0.02	6.0
109	25	10	0.02	6.1
110	26	10	0.02	6.1
111	27	10	0.02	6.1
112	28	10	0.02	6.1
113	29	10	0.02	6.1
114	30	10	0.02	6.1
115	25	10	0.02	6.2
116	26	10	0.02	6.2
117	27	10	0.02	6.2
118	28	10	0.02	6.2
119	29	10	0.02	6.2
120	30	10	0.02	6.2
121	25	10	0.02	6.3
122	26	10	0.02	6.3

123	27	10	0.02	6.3
124	28	10	0.02	6.3
125	29	10	0.02	6.3
126	30	10	0.02	6.3

In some embodiments, formulation (ii) further comprises 5-30 mM arginine-HCl, preferably 6-25 mM arginine-HCl, more preferably 7-20 mM arginine-HCl, more preferably 8-15 mM arginine-HCl, more preferably 9-12 mM arginine-HCl, and most preferably 10 mM arginine-HCl. In principle, any pharmaceutically acceptable arginine-HCl may be used, and the arginine-HCl may be L-arginine-HCl, D-arginine-HCl or a mixture of D- and L-arginine-HCl. However, in a preferred embodiment the arginine-HCl is L-arginine-HCl, as this is considered to be most natural and possibly least immunogenic.

In addition to arginine-HCl, formulation (ii) may additionally comprise 0.5-10 mM methionine, preferably 0.75-5 mM methionine, more preferably 1-3 mM methionine, more preferably 1.5-2.5 mM methionine, and most preferably 2 mM methionine. In principle, any pharmaceutically acceptable methionine may be used, and the methionine may be L-methionine, D- methionine or a mixture of D- and L- methionine. However, in a preferred embodiment the methionine is L- methionine, as this is considered to be most natural and possibly least immunogenic.

In still further embodiments, formulation (ii) may further comprise 10-100 mM NaCl, preferably 20-80 mM NaCl, more preferably 25-75 mM NaCl, more preferably 30-70 mM NaCl, more preferably 40-60 mM NaCl, more preferably 45-55 mM NaCl, and most preferably 50 mM NaCl.

Very preferred embodiments of formulation (ii) of the present disclosure consists of

- 25-30 mM sodium phosphate buffer, 5 % (w/v) sorbitol, and 0.02 % (w/v) polysorbate 80, pH 6.5.
- 25-30 mM sodium phosphate buffer, 5 % (w/v) sorbitol, 50 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7-6.5.
- 25-30 mM sodium phosphate buffer, 5 % (w/v) sorbitol, 50 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.
- 25-30 mM sodium phosphate buffer, 5 % (w/v) sorbitol, 10 mM L-arginine-HCl, and 0.02 % (w/v) polysorbate 80, pH 6.1.
- 25-30 mM sodium phosphate buffer, 5 % (w/v) sorbitol, 10 mM L-arginine-HCl, 2 mM L-methionine, and 0.02 % (w/v) polysorbate 80, pH 6.1.
- 30 mM sodium phosphate buffer, 5 % (w/v) sorbitol, and 0.02 % (w/v) polysorbate 80, pH 6.5.
- 30 mM sodium phosphate buffer, 5 % (w/v) sorbitol, 50 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7-6.5.

- 30 mM sodium phosphate buffer, 5 % (w/v) sorbitol, 50 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.
- 30 mM sodium phosphate buffer, 5 % (w/v) sorbitol, 10 mM L-arginine-HCl, and 0.02 % (w/v) polysorbate 80, pH 6.1.
- 30 mM sodium phosphate buffer, 5 % (w/v) sorbitol, 10 mM L-arginine-HCl, 2 mM L-methionine, and 0.02 % (w/v) polysorbate 80, pH 6.1.

In a most preferred embodiment, said formulation (i) is 25-30 mM (most preferably 30 mM) sodium phosphate buffer, 5 % (w/v) sorbitol, and 0.02 % (w/v) polysorbate 80, pH 6.5.

Formulation (iii) comprises, preferably consists of, 1-50 mg/ml (preferably 5-50 mg/ml, more preferably 10-45 mg/ml, even more preferably 15-40 mg/ml, still more preferably 20-30 mg/ml, and most preferably 20 mg/ml) of a human or humanized full-length IgG_4 as further disclosed above, and 10-25 mM histidine buffer, 10-50 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant of a surfactant as further disclosed above, pH 5.1-6.5. In some embodiments, formulation (iii) comprises 10-20 mM histidine buffer; preferably 10-15 mM histidine buffer, more preferably 10-12 mM histidine buffer, and most preferably 10 mM histidine buffer. In principle, any pharmaceutically acceptable histidine buffer may be used, and the histidine may be L-histidine, D-histidine or a mixture of D- and L-histidine. However, in a preferred embodiment the histidine is L-histidine, as this is considered to be most natural and possibly least immunogenic. It further embodiments, formulation (iii) comprises 15-40 mM sodium chloride, preferably 20-30 mM sodium chloride, more preferably 20-25 mM sodium chloride, and most preferably 20 mM sodium chloride. The pH of formulation (iii) is generally in the range of 5.1-6.5, while preferred embodiments are wherein in formulation (iii) pH is 5.1-6.4, preferably 5.1-6.3, more preferably 5.1-6.2, more preferably 5.1-6.1, more preferably 5.2-6.0, more preferably pH is 5.3-5.9, more preferably pH is 5.4-5.8, even more preferably pH is 5.5-5.7. Most preferably formulation (iii) has a pH of 5.7.

As shown in the examples, in certain embodiments it can be advantageous to add very low amounts of a phosphate buffer in addition to the histidine buffer. Hence, in some embodiments, formulation (iii) further comprises 1-5 mM of a phosphate buffer, preferably 2-4 mM of a phosphate buffer, more preferably 2.5-3 mM of a phosphate buffer, and most preferably 2.5 mM of a phosphate buffer. While in principle any pharmaceutically acceptable phosphate buffer may be used, it is preferred that the phosphate buffer of formulation (iii) of the present disclosure is sodium phosphate buffer, e.g., in order to avoid administration of potassium ions which can affect heart function.

In some embodiments, formulation (iii) further comprises 5-30 mM methionine, preferably 6-25 mM methionine, more preferably 7-20 mM methionine, more preferably 8-15 mM methionine, more preferably 9-12 mM methionine, and most preferably 10 mM

methionine. In principle, any pharmaceutically acceptable methionine may be used, and the methionine may be L- methionine, D- methionine or a mixture of D- and L- methionine. However, in a preferred embodiment the methionine is L- methionine, as this is considered to be most natural and possibly least immunogenic.

In addition to methionine, formulation (iii) may additionally comprises 5-50 mM glycine, preferably 10-45 mM glycine, more preferably 15-40 mM glycine, more preferably 20-35 mM glycine, more preferably 25-30 mM glycine, and most preferably 30 mM glycine.

In still further embodiments, formulation (iii) may further comprise 5-10 % (w/v) of a polyol, preferably 5-9 % (w/v) of a polyol, more preferably 5-8 % (w/v) of a polyol, more preferably 5-7 % (w/v) of a polyol, more preferably 5-6 % (w/v) of a polyol, and most preferably 5 % (w/v) of a polyol. In principle, any pharmaceutically acceptable polyol may be used. However, in preferred embodiments, the polyol is selected from sorbitol, sucrose, mannitol, xylitol, and inositol; more preferably wherein the polyol is sorbitol or sucrose, and most preferably the polyol is sorbitol. Accordingly, in one preferred embodiments, formulation (iii) further comprises 5 % (w/v) of sorbitol.

Very preferred embodiments of formulation (iii) of the present disclosure consists of

- 10 mM L-histidine buffer, 20 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.
- 10 mM L-histidine buffer, 20 mM NaCl, 5 % (w/v) of sorbitol, and 0.02 % (w/v) polysorbate 80, pH 5.7.
- 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 20 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.
- 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 20 mM NaCl, 10mM L-methionine, and 0.02 % (w/v) polysorbate 80, pH 5.7.
- 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 20 mM NaCl, 10mM L-methionine, 30 mM glycine, and 0.02 % (w/v) polysorbate 80, pH 6.1.

The conductivity of a formulation correlates with the presence of ions in the formulation. A higher conductivity can be advantageous depending on the molecules and its surface charge distribution. In the present case, without wishing to be bound by theory, it is assumed that a higher conductivity will break long range surface charge-charge interactions in the human or humanized IgG_4. These long range surface charge-charge interactions in proteins are known to lead to a higher aggregation. Therefore, in a preferred embodiment, the conductivity of the pharmaceutical composition of the present disclosure is at least 2.5 mS/cm, preferably at least 2.6 mS/cm. Consequently, in formulations like formulation (ii) described herein which do not contain salts such as sodium chloride, stability of the human or humanized IgG_4 is increased by increasing the buffer concentration. On the other hand, the presence of too many charged ions in the solution may lead to charge screening and a salting-out effect, which shall be avoided. Consequently, in formulations like formulation (i) which contain sodium chloride, stability

of human or humanized IgG_4 is improved, since the buffer concentration is rather kept low. It is believed that within the indicated concentration ranges for the pharmaceutical compositions no salting-out effects are observed, for which reasons it is not necessary to indicate an upper limit for the conductivity.

Accordingly, in view of the foregoing, the present disclosure also provides a method for stabilizing a human or humanized full length IgG_4, comprising combining 1-50 mg/ml (preferably 5-50 mg/ml, more preferably 10-45 mg/ml, even more preferably 15-40 mg/ml, still more preferably 20-30 mg/ml, and most preferably 20 mg/ml) of a human or humanized full-length IgG_4 with a formulation selected from

(i) a formulation comprising 10-25 mM histidine buffer, 130-180 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5; or

(ii) a formulation comprising 25-30 mM phosphate buffer, 5-10 % (w/v) of a polyol, and 0.001-0.5 % (w/v) of a surfactant, pH 5.0-6.9; or

(iii) a formulation comprising 10-25 mM histidine buffer, 10-50 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5;

thereby preparing a stable aqueous pharmaceutical composition (i)-(iii) as described above. Preferred embodiments of the formulations (i)-(iii) added to the human or humanized full-length IgG_4 are as further disclosed for formulations (i)-(iii) above. As also noted above, it is preferred that the IgG_4 is a humanized IgG_4, in particular wherein the human or humanized full length IgG_4 is essentially identical / similar to natalizumab, more preferably wherein the IgG_4 is natalizumab, as further disclosed in detail above. In addition, it is also preferred that the final aqueous pharmaceutical composition has a conductivity of at least 2.5 mS/cm, preferably at least 2.6 mS/cm for the above indicated reasons.

Likewise, the present disclosure also provides a method for producing an aqueous pharmaceutical composition (i)-(iii) as described above, comprising combining 1-50 mg/ml (preferably 5-50 mg/ml, more preferably 10-45 mg/ml, even more preferably 15-40 mg/ml, still more preferably 20-30 mg/ml, and most preferably 20 mg/ml) of a human or humanized full length IgG_4 with a formulation selected from

(i) a formulation comprising 10-25 mM histidine buffer, 130-180 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5; or

(ii) a formulation comprising 25-30 mM phosphate buffer, 5-10 % (w/v) of a polyol, and 0.001-0.5 % (w/v) of a surfactant, pH 5.0-6.9; or

(iii) a formulation comprising 10-25 mM histidine buffer, 10-50 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5.

Preferred embodiments of the formulation (i)-(iii) added to the human or humanized full-length IgG_4 are as further disclosed above. As also noted above, it is preferred that the

IgG_4 is a humanized IgG_4, in particular wherein the human or humanized full length IgG_4 is essentially identical / similar to natalizumab, more preferably wherein the IgG_4 is natalizumab, as further disclosed in detail above. In addition, it is also preferred that the final aqueous pharmaceutical composition has a conductivity of at least 2.5 mS/cm, preferably at least 2.6 mS/cm for the above indicated reasons.

The present disclosure further provides an article of manufacture, comprising a pharmaceutical composition of the present disclosure and instructions for use of said composition. In preferred embodiments, said pharmaceutical composition is contained in a pre-filled vial or in a pre-filled syringe, wherein it is more preferred that said pharmaceutical composition is contained in a pre-filled vial. Suitable vials and syringes for pharmaceutical purposes, e.g., those described in the examples section below, are known and used in the field, and can be obtained from commercial suppliers.

Since the pharmaceutical compositions are intended for in vivo administration, in particular by infusion, it is clear to the skilled person that the final pharmaceutical compositions are required to be sterile. The pharmaceutical composition may be administered at a suitable dose, i.e. about 1 ng/kg body weight to about 100 mg/kg body weight of a subject, preferably at a dose of about 10 ng/kg to about 10 mg/kg, more preferably at a dose of about 10 ng/kg to about 5 mg/kg per body weight. However, the dosage regimen will be determined by an attending physician and depend upon many factors, including the therapeutic IgG_4 to be administered, patient's size and condition, body surface area, age, sex, time and route of administration, and on other drugs being administered concurrently. Administration will preferably be intravenously but may also be subcutaneously, intramuscularly, or intraperitoneally.

Natalizumab is marketed by Biogen MA, Inc. under the name Tysabri, and has FDA-approval for the treatment of multiple sclerosis and Crohn's disease, and EMEA approval for the treatment of multiple sclerosis. Recently, it was suggested that natalizumab could also be used in a combination treatment of B-cell malignancies, where it is intended to overcome the resistance to rituximab. Accordingly, it is to be expected that the pharmaceutical composition of the present disclosure can be suitably applied in the treatment of multiple sclerosis or Crohn's disease, and possibly in the treatment of B-cell malignancies. Administration of natalizumab may be carried out as follows: 15 mL concentrate from the vial will be extracted and diluted in 100 mL 0.9% NaCl. The bag will be gently inverted, but not agitated. After dilution, the solution will be used immediately or within 8 hr (keep refrigerated at 2-8°C, do not freeze it). The infusion of equilibrated to room temperature solution will be done in 1 hour, followed by a flash with 0,9% NaCl.

The invention is further described by the following embodiments.

1. An aqueous pharmaceutical composition comprising 1-50 mg/ml of a human or humanized full-length IgG_4 in a formulation selected from
   (i) a formulation comprising 10-25 mM histidine buffer, 130-180 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5; or
   (ii) a formulation comprising 25-30 mM phosphate buffer, 5-10 % (w/v) of a polyol, and 0.001-0.5 % (w/v) of a surfactant, pH 5.0-6.9; or
   (iii) a formulation comprising 10-25 mM histidine buffer, 10-50 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5.
2. The pharmaceutical composition of embodiment 1, wherein said pharmaceutical composition comprises 5-50 mg/ml, preferably 10-45 mg/ml, more preferably 15-40 mg/ml, even more preferably 20-30 mg/ml of said human or humanized full-length IgG_4.
3. The pharmaceutical composition of embodiment 1 or 2, wherein the IgG_4 is a humanized IgG_4.
4. The pharmaceutical composition of any one of embodiments 1-3, wherein the human or humanized full-length IgG_4 is natalizumab.
5. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation comprises 0.005-0.1 % (w/v), preferably 0.01-0.05 % (w/v), and more preferably 0.02 % (w/v) surfactant.
6. The pharmaceutical composition of any one of embodiments 1-5, wherein the surfactant is a nonionic surfactant.
7. The pharmaceutical composition of embodiment 6, wherein the nonionic surfactant is selected from polysorbate (Tween), poloxamer (Pluronic), polyethylene glycol alkyl ether (Brij), polyethylene glycol octylphenyl ether (Triton X-100), polypropylene glycol alkyl ether, glucoside alkyl ether, glycerol alkyl ester, and dodecyldimethylamine oxide; preferably wherein the nonionic surfactant is polysorbate or poloxamer; more preferably wherein the nonionic surfactant is polysorbate 80, polysorbate 20, or poloxamer 188.
8. The pharmaceutical composition of embodiment 6, wherein the nonionic surfactant is polysorbate, preferably, wherein the nonionic surfactant is polysorbate 80.
9. The pharmaceutical composition of any one of embodiments 1-8, wherein formulation (i) comprises 10-20 mM histidine buffer; preferably 10-15 mM histidine buffer, more preferably 10-12 mM histidine buffer, and most preferably 10 mM histidine buffer.
10. The pharmaceutical composition of any one of embodiments 1-9, wherein said histidine in formulation (i) is L-histidine.
11. The pharmaceutical composition of any one of embodiments 1-10, wherein formulation (i) comprises 140-170 mM sodium chloride, preferably 145-165 mM sodium chloride, more preferably 150-160 mM sodium chloride, and most preferably 150 mM sodium chloride.
12. The pharmaceutical composition of any one of embodiments 1-11, wherein in formulation (i) the pH is 5.1-6.4, preferably pH is 5.1-6.3, more preferably the pH is

5.1-6.2, more preferably the pH is 5.1-6.1, more preferably pH is 5.2-6.0, more preferably pH is 5.3-5.9, more preferably pH is 5.4-5.8, even more preferably pH is 5.5-5.7, and most preferably formulation (i) has a pH of 5.7.

13. The pharmaceutical composition of any one of embodiments 1-12, wherein formulation (i) further comprises 5-30 mM methionine, preferably 6-25 mM methionine, more preferably 7-20 mM methionine, more preferably 8-15 mM methionine, more preferably 9-12 mM methionine, and most preferably 10 mM methionine.
14. The pharmaceutical composition of embodiment 13, wherein the methionine is L-methionine.
15. The pharmaceutical composition of any one of embodiments 13 or 14, wherein formulation (i) further comprises 5-50 mM glycine, preferably 10-45 mM glycine, more preferably 15-40 mM glycine, more preferably 20-35 mM glycine, more preferably 25-30 mM glycine, and most preferably 30 mM glycine.
16. The pharmaceutical composition of any one of embodiments 1-15, wherein formulation (i) further comprises 1-5 mM of a phosphate buffer, preferably 2-4 mM of a phosphate buffer, more preferably 2.5-3 mM of a phosphate buffer, and most preferably 2.5 mM of a phosphate buffer.
17. The pharmaceutical composition of embodiment 16, wherein said phosphate buffer is sodium phosphate buffer.
18. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (i) consists of 10 mM L-histidine buffer, 150 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.
19. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (i) consists of 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 150 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.
20. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (i) consists of 25 mM L-histidine buffer, 150 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.
21. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (i) consists of 10 mM L-histidine buffer, 150 mM NaCl, 10mM L-methionine, and 0.02 % (w/v) polysorbate 80, pH 5.7.
22. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (i) consists of 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 150 mM NaCl, 10mM L-methionine, and 0.02 % (w/v) polysorbate 80, pH 5.7.
23. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (i) consists of 25 mM L-histidine buffer, 150 mM NaCl, 10mM L-methionine, and 0.02 % (w/v) polysorbate 80, pH 5.7.
24. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (i) consists of 10 mM L-histidine buffer, 150 mM NaCl, 10mM L-methionine, 30 mM glycine, and 0.02 % (w/v) polysorbate 80, pH 6.1.

25. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (i) consists of 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 150 mM NaCl, 10mM L-methionine, 30 mM glycine, and 0.02 % (w/v) polysorbate 80, pH 6.1.
26. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (i) consists of 25 mM L-histidine buffer, 150 mM NaCl, 10mM L-methionine, 30 mM glycine, and 0.02 % (w/v) polysorbate 80, pH 6.1.
27. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (i) consists of 10 mM L-histidine buffer, 150 mM NaCl, and 0.2 % (w/v) polysorbate 80, pH 5.7.
28. The pharmaceutical composition of any one of embodiments 1-8, wherein in formulation (ii) said phosphate buffer is sodium phosphate buffer.
29. The pharmaceutical composition of any one of embodiments 1-8 or 28, wherein the polyol in formulation (ii) is selected from sorbitol, sucrose, mannitol, xylitol, and inositol; preferably wherein the polyol is sorbitol or sucrose, more preferably wherein the polyol is sorbitol.
30. The pharmaceutical composition of any one of embodiments 1-8 or 28-29, wherein formulation (ii) comprises 5 % (w/v) of said polyol.
31. The pharmaceutical composition of any one of embodiments 1-8 or 28-30, wherein in formulation (ii) pH is 5.5-6.8, preferably 5.7-6.7, more preferably 5.8-6.7, more preferably 5.8-6.5, more preferably 6.0-6.5, and most preferably a pH of 6.1-6.3.
32. The pharmaceutical composition of any one of embodiments 1-8 or 28-31, wherein formulation (ii) further comprises 5-30 mM arginine-HCl, preferably 6-25 mM arginine-HCl, more preferably 7-20 mM arginine-HCl, more preferably 8-15 mM arginine-HCl, more preferably 9-12 mM arginine-HCl, and most preferably 10 mM arginine-HCl.
33. The pharmaceutical composition of embodiment 32, wherein the arginine-HCl is L-arginine-HCl.
34. The pharmaceutical composition of any one of embodiments 32 or 33, wherein formulation (ii) further comprises 0.5-10 mM methionine, preferably 0.75-5 mM methionine, more preferably 1-3 mM methionine, more preferably 1.5-2.5 mM methionine, , and most preferably 2 mM methionine.
35. The pharmaceutical composition of embodiment 34, wherein the methionine is L-methionine.
36. The pharmaceutical composition of any one of embodiments 1-8 or 28-31, wherein formulation (ii) further comprises 10-100 mM NaCl, preferably 20-80 mM NaCl, more preferably 25-75 mM NaCl, more preferably 30-70 mM NaCl, more preferably 40-60 mM NaCl, more preferably 45-55 mM NaCl, and most preferably 50 mM NaCl.
37. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (ii) consists of 25-30 mM sodium phosphate buffer, 5 % (w/v) sorbitol, and 0.02 % (w/v) polysorbate 80, pH 6.5.

38. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (ii) consists of 25-30 mM sodium phosphate buffer, 5 % (w/v) sorbitol, 50 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7-6.5.
39. The pharmaceutical composition of embodiment 38, wherein the pH is 5.7.
40. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (ii) consists of 25-30 mM sodium phosphate buffer, 5 % (w/v) sorbitol, 10 mM L-arginine-HCl, and 0.02 % (w/v) polysorbate 80, pH 6.1.
41. The pharmaceutical composition of embodiment 40, further comprising 2 mM L-methionine.
42. The pharmaceutical composition of any one of embodiments 37-40, wherein formulation (ii) comprises 30 mM sodium phosphate.
43. The pharmaceutical composition of any one of embodiments 1-8, wherein formulation (iii) comprises 10-20 mM histidine buffer; preferably 10-15 mM histidine buffer, more preferably 10-12 mM histidine buffer, and most preferably 10 mM histidine buffer.
44. The pharmaceutical composition of any one of embodiments 1-8 or 43, wherein said histidine in formulation (iii) is L-histidine.
45. The pharmaceutical composition of any one of embodiments 1-8 or 43-44, wherein formulation (iii) comprises 15-40 mM sodium chloride, preferably 20-30 mM sodium chloride, more preferably 20-25 mM sodium chloride, and most preferably 20 mM sodium chloride.
46. The pharmaceutical composition of any one of embodiments 1-8 or 43-45, wherein in formulation (iii) the pH is 5.1-6.4, preferably pH is 5.1-6.3, more preferably the pH is 5.1-6.2, more preferably the pH is 5.1-6.1, more preferably pH is 5.2-6.0, more preferably pH is 5.3-5.9, more preferably pH is 5.4-5.8, even more preferably pH is 5.5-5.7, and most preferably formulation (iii) has a pH of 5.7.
47. The pharmaceutical composition of any one of embodiments 1-8 or 43-46, wherein formulation (iii) further comprises 1-5 mM of a phosphate buffer, preferably 2-4 mM of a phosphate buffer, more preferably 2.5-3 mM of a phosphate buffer, and most preferably 2.5 mM of a phosphate buffer.
48. The pharmaceutical composition of embodiment 47, wherein said phosphate buffer is sodium phosphate buffer.
49. The pharmaceutical composition of any one of embodiments 1-8 or 43-48, wherein formulation (iii) further comprises 5-30 mM methionine, preferably 6-25 mM methionine, more preferably 7-20 mM methionine, more preferably 8-15 mM methionine, more preferably 9-12 mM methionine, and most preferably 10 mM methionine.
50. The pharmaceutical composition of embodiment 49, wherein the methionine is L-methionine.
51. The pharmaceutical composition of any one of embodiments 49 or 50, wherein formulation (iii) further comprises 5-50 mM glycine, preferably 10-45 mM glycine,

more preferably 15-40 mM glycine, more preferably 20-35 mM glycine, more preferably 25-30 mM glycine, and most preferably 30 mM glycine.

52. The pharmaceutical composition of any one of embodiments 1-8 or 43-46, wherein formulation (iii) further comprises 5-10 % (w/v) of a polyol, preferably 5-9 % (w/v) of a polyol, more preferably 5-8 % (w/v) of a polyol, more preferably 5-7 % (w/v) of a polyol, more preferably 5-6 % (w/v) of a polyol, and most preferably 5 % (w/v) of a polyol.

53. The pharmaceutical composition of embodiment 52, wherein the polyol is selected from sorbitol, sucrose, mannitol, xylitol, and inositol; preferably wherein the polyol is sorbitol or sucrose, more preferably wherein the polyol is sorbitol.

54. The pharmaceutical composition of embodiment 53, wherein formulation (iii) comprises 5 % (w/v) of sorbitol.

55. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (iii) consists of 10 mM L-histidine buffer, 20 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.

56. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (iii) consists of 10 mM L-histidine buffer, 20 mM NaCl, 5 % (w/v) of sorbitol, and 0.02 % (w/v) polysorbate 80, pH 5.7.

57. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (iii) consists of 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 20 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.

58. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (iii) consists of 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 20 mM NaCl, 10mM L-methionine, and 0.02 % (w/v) polysorbate 80, pH 5.7.

59. The pharmaceutical composition of any one of embodiments 1-4, wherein said formulation (iii) consists of 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 20 mM NaCl, 10mM L-methionine, 30 mM glycine, and 0.02 % (w/v) polysorbate 80, pH 6.1.

60. The pharmaceutical composition of any one of embodiments 1-59, wherein the conductivity of said pharmaceutical composition is at least 2.5 mS/cm, preferably at least 2.6 mS/cm.

61. An article of manufacture, comprising a pharmaceutical composition according to any one of embodiments 1-60 and instructions for use of said composition, preferably wherein said pharmaceutical composition is contained in a pre-filled vial or in a pre-filled syringe, more preferably wherein said pharmaceutical composition is contained in a pre-filled vial.

62. A method for stabilizing a human or humanized full length IgG_4, comprising combining 1-50 mg/ml of a human or humanized full-length IgG_4 with a formulation selected from

(i) a formulation comprising 10-25 mM histidine buffer, 130-180 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5; or

(ii) a formulation comprising 25-30 mM phosphate buffer, 5-10 % (w/v) of a polyol, and 0.001-0.5 % (w/v) of a surfactant, pH 5.0-6.9; or

(iii) a formulation comprising 10-25 mM histidine buffer, 10-50 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5;

thereby preparing a stable aqueous pharmaceutical composition.

63. A method for producing an aqueous pharmaceutical composition according to any one of embodiments 1-60, comprising combining 1-50 mg/ml of a human or humanized full length IgG_4 with a formulation selected from

(i) a formulation comprising 10-25 mM histidine buffer, 130-180 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5; or

(ii) a formulation comprising 25-30 mM phosphate buffer, 5-10 % (w/v) of a polyol, and 0.001-0.5 % (w/v) of a surfactant, pH 5.0-6.9; or

(iii) a formulation comprising 10-25 mM histidine buffer, 10-50 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5.

64. The method of embodiment 62 or embodiment 63, wherein the human or humanized full-length IgG_4 is added in an amount of 5-50 mg/ml, preferably 10-45 mg/ml, more preferably 15-40 mg/ml, even more preferably 20-30 mg/ml of said human or humanized full-length IgG_4.

65. The method of any one of embodiments 62-64, wherein the IgG_4 is a humanized IgG_4.

66. The method of any one of embodiments 62-65, wherein the human or humanized full-length IgG_4 is natalizumab.

67. The method of any one of embodiments 62-66, wherein the formulation added to the human or humanized full-length IgG_4 is as further defined in embodiments 5-59.

68. The method of any one of embodiments 62-67, wherein the final stable aqueous pharmaceutical composition has a conductivity of at least 2.5 mS/cm, preferably at least 2.6 mS/cm.

69. An aqueous pharmaceutical composition according to any one of embodiments 1-60 for use in the treatment of multiple sclerosis or Crohn's disease.

In the following, the present invention as defined in the embodiments is further illustrated by the following examples, which are not intended to limit the scope of the present invention. All references cited herein are explicitly incorporated by reference.

DESCRIPTION OF THE SEQUENCES

The start and stop codons are indicated in bold, the signal sequence is underlined.

Natalizumab Light Chain

Atgaagtgggtgaccttcatctccctgctgtttctgttctcctccgcctactccgacatccagatgacccagt
ccccctccagcctgtccgcctccgtgggcgacagagtgaccatcacatgcaagacctcccaggacatcaacaa
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agccccgtgaccaagtccttcaaccggggcgagtgc**tgatag** (SEQ ID NO: 1)

Translation

MKWVTFISLLFLFSSAYSDIQMTQSPSSLSASVGDRVTITCKTSQDINKYMAWYQQTPGKAPRLLIHYTSALQ
PGIPSRFSGSGSGRDYTFTISSLQPEDIATYYCLQYDNLWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS
SPVTKSFNRGEC (SEQ ID NO: 2)

Natalizumab Heavy Chain

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ccctgagcctgggcaag**tgatag** (SEQ ID NO: 3)

Translation

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GYTKYDPKFQGRVTITADTSASTAYMELSSLRSEDTAVYYCAREGYYGNYGVYAMDYWGQGTLVTVSSASTKG
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KTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDP
EVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPR
EPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 4)

EXAMPLES

Example 1

The initial screening of excipients was carried out using techniques that target colloidal and conformational stability of IgG_4 in various conditions using Differential Scanning Calorimetry (DSC) and Composition gradient multi-angle light scattering (CG-MALS). A sample of 20 mg/ml Natalizumab IgG_4 antibody obtained from a 10L batch, produced at R&D DSP of Polpharma Biologic (PB006_Batch1) was used as the IgG_4 antibody.

DSC is specifically designed to determine the denaturation temperature and denaturation enthalpy of proteins and other macromolecules in solution, i.e. the amount of heat that is absorbed or released from biomolecules in solution during heating or cooling, with the versatility and precision to perform molecular stability screenings. It is a tool of choice for conformational stability. Native proteins respond to heating by unfolding (thermal denaturation) at a characteristic temperature referred to as melting temperature (T_m). The more intrinsically stable the biopolymer is the higher is the temperature of the unfolding transition (T_m) and the transition enthalpy. The experiment was performed in scanning mode in temperature range of 20°C to 100°C with heating and scanning rate of 1 °C/min. A buffer scan was conducted prior to each sample run to generate a baseline. Samples were prepared via dialysis against the corresponding formulation buffer and diluted to about 1 mg/mL. The corresponding dialysis buffer was used as buffer scan. The exact sample concentration after dialysis was determined by UV (280 nm) absorption.

An interaction between protein molecules in solution was characterized by changes in their light scattering behavior at different concentrations via CG-MALS. CG-MALS is a well-established technique that addresses colloidal stability of the biomolecule by calculation of the second viral coefficient A_2 that describes molecule interactions in Newtonian's solutions. The characteristic parameter to measure molecule interactions is the second virial coefficient A_2. A_2 is characteristic for a macromolecule and its solvent, and describes molecular interactions between the dissolved macromolecules. A negative A_2 indicates attractive interactions whereas a positive A_2 is characteristic for repulsive interactions between the dissolved molecules.

More specifically, the CG-MALS technique is based on a concentration series of a macromolecular solution which are directly injected into the flow cell of a multi angle light scattering detector. After each injection the flow is stopped to permit the reaction to reach equilibrium. The apparent molecular weight (Mw_{app}) is determined for each step in the gradient by analyzing light scattering and concentration data by the following equation:

$$Mw_{app} \models \frac{R(\theta,c)}{K \times c}$$

wherein

Mw_{app}: apparent molecular weight

R(θ,c) **:** excess Rayleigh ratio of the solution as a function of scattering angle θ and concentration c. It is directly proportional to the intensity of the excess light scattered by the solute and the light scattered by the pure solvent.

K: Optical constant ($4p^2(dn/dc)^2n_0^2/N_al_0^4$)

c: Concentration

Significant interactions between macromolecules manifest as changes in Mw_{app} vs. concentration.

A_2 calculation was conducted via Zimm plot analysis according to:

$$\frac{K \cdot c}{R(\theta,c)} = \frac{1}{M_w P(\theta)} + A_2 c$$

wherein

R(θ,c) **:** excess Rayleigh ratio of the solution as a function of scattering angle θ and concentration c. It is directly proportional to the intensity of the excess light scattered by the solute and the light scattered by the pure solvent.

Mw: Weight average molecular weight

A2: 2nd virial coefficient

c: Concentration

K: Optical constant ($4p^2(dn/dc)^2n_0^2/N_al_0^4$)

P(θ): describes the angular dependence of the scattered light, and can be related to the rms radius. Below rms radii of 30 nm this term becomes 0.

The following selection of formulations F1-F4 were nominated for further analysis. Formulation F5 is provided for comparison and represents the current commercial formulation of natalizumab. The formulations were also evaluated for buffering capacity, conductivity and osmolality.

Table 1. Selected formulations based on excipients, buffers and pH screening

#	pH	buffer substance	stabilizer	T_{onset} °C	T_{max} °C	A_2 10^{-}	λ mS/cm	Osmolality mOsm/kg

						4 $mol*mL*g^{-}$ 2		
F1	5,7	10 mM His/HCl	150 mM NaCl	61,6	81,0	-0,102	15,9	290
F2	6,1	29 mM Na-phosphate	500 mM Proline	61,6	82,0	-0,008	2,62	733
F3	5,7	39 mM Na acetate	250 mM Proline	61,3	81,9	-0,005	2,61	373
F4	6,5	26 mM Na-phosphate	5% (w/v) Sorbitol	65,2	82,3	-0,224	2,63	340
F5	6.1	10 mM Na-Phosphate	140 mM NaCl	62.8	81.4	-0.125	15.8	281

Example 2

Based on the results presented in Table 1 F1 and F4 were selected for further analysis during forced degradation studies. Formula F1 shows a good compromise between a high conductivity and A_2, and formula 4 exhibits a high T_{onset} value. The requirement for conductivity of more than 2.5 mS/cm and isotonicity (250-350 mOsm/kg) were also considered during the selection of formula F1.

F1 and F4 were tested for various stresses including shaking in elevated temperature and freezing and thawing and compared to the reference formulation F5.

The shaking studies were carried at 200 rpm at 40 °C for 0.9 mL of 20 mg/ml of natalizumab batch PB006_Batch2 in 2R vials over 1 d, 3 d and 6 d. Samples were analyzed using size exclusion chromatography (SEC). SEC is a chromatographic technique that allows separation of monomeric forms from aggregated species present in biologics samples. The assay is performed using UPLC system equipped with Waters BEH200 SEC 1.7 um 4.6x150 mm at column temperature 30+/-0.5°C. The mobile phase used was 100 mM sodium phosphate buffer with 200 mM sodium chloride pH 6.8+/- 0.05 at a flow rate of 0.3 ml/min. The samples were diluted to 1 mg/ml in mobile phase and then a volume of 2 µl was injected to the system. The measurement was performed at 210 nm. The SEC data after 6 days of shaking stress showed no significant differentiations between samples.

Freeze-thaw stress for liquid samples was performed in 2R vials (1.0 mL fill, 20 mg/ml of natalizumab PB006_Batch2). The samples were exposed to three cycles of freezing and thawing. Samples were frozen from room temperature to -80 °C for 8 hr and then thawed for 2 hr at room temperature. The data from DSC for the two selected formulations F1 and F4 is provided in the below table.

Table 2. DSC data summary. Tm1 results for selected formulations, F1, F4 and F5.

	T_m 1(°C)	
	T0	**F/T**
F1	67,52	67,8
F4	70,46	70,44
F5	68,47	68,56

The data from DSC shows conformational stability, since both formulations F1 and F4 have their T_{m1} (CH_2 domain unfolding) in the range from 65°C to 70°C. In particular formulation F4 confers a slightly increased conformational stability to natalizumab.

In the second part of the experiment, liquid samples in 2R vials (1,0 mL fill, 20 mg/ml of natalizumab batch PB006_Batch2) were exposed to three cycles of freezing and thawing. Samples were frozen from room temperature to -80 °C for 8 hr and then thawed for 2 hr at room temperature. Samples were subjected to microflow imaging (MFI) before and after the three freeze-thaw-cycles.

MFI is a technique that allows characterizing sub-visible particles in the range of 2µm-200µm in a given formulation. Before sample preparation, the system was primed with related formulation buffer to optimize illumination first. Samples were prepared by gently mixing same in circular motions after removal from storage condition. Then the vials were opened and 1 ml of sample was withdrawn from a vial using pipette with 1 ml tip with filter. The tip, then was placed in the inlet (dispensing) port of the MFI device and tested. The sample volume of 1ml was used to cover 0.1 ml for dead volume and 0.9 ml for dispensing. Following parameters were set on for data acquisition:

Sample dispensed: 0.9 ml

Purge volume: 0.2ml

Sample analyzed volume:0.59 ml

Edge particles rejection: Enabled

Fill particles: Enabled

The data was analyzed by setting data filters above 10 µm. The results are shown in the following table.

Table 3. MFI data summary sub-visible particle count for the range above 10 nm. Results for selected formulations, F1, F4 and F5. The data indicates slightly increased stability for F4 and F1 formulations.

	Sub-visible particles (>10 µm)	
	T0	**F/T**
F1	5	29
F4	23	13
F5	29	46

Example 3

In light of the foregoing results, the following formulations are tested for long term stability at various temperatures 5°C, 25°C, and 40°C ,and selected time points for -70°C. The material for the study was produced at Polpharma Biologics (PB006_Batch3).

Drug Substance (DS) formulated in 10 mM Sodium Phosphate, 140 mM Sodium Chloride, 0.02% polysorbate 80, pH 6.1, was filled by Polpharma Biologics into 20L Meissner 2D end-ported Biocontainers (TPE) (Part number: B12E00203-005)from a 250L pilot-plant scale batch of PB006_Batch3 product, and was stored at 2-8°C in controlled and monitored conditions until use in the stability study.

For the stability study, the material was divided into 3 equal fractions. Then the fractions were dialyzed against the respective formulation buffers that were selected for the stability studies (cf. Table 4).

Table 4. List of formulation buffers used in Long Term Stability studies.

#	pH	Buffer substance	Stabilizer	Surfactant
F1	5.7	10 mM His/HCl	150 mM NaCl	0.02%(w/v) Polysorbate 80
F4	6.5	30 mM Na-Phosphate	5% (w/v) Sorbitol	0.02%(w/v) Polysorbate 80
F5	6.1	10 mM Na-Phosphate	140 mM NaCl	0.02%(w/v) Polysorbate 80

Following buffer exchange, concentration of the samples was tested and met the target concentration of 19-21 mg/ml.

The sample vials for the stability studies were prepared under aseptic conditions in a laminal flow hood by manual filling and sealing of the sample into 15R vials. Details of primary packaging are provided in Table 5.

Table 5. Details of primary packaging used in the Long Term Stability studies.

Vial	15R fiolax glass (type 1B), inner coating: baked silicon
Stopper	20 mm bromobutyl rubber FM457, coated with Omniflex 3G (fluoropolymer)
Seal	20mm crimp flip-off seal of aluminum foil with a blue plastic button without embossed flip-off sign
Container Quantity	15 ml (300 mg/vial)

The packed vials were then labeled and placed under appropriate stability conditions for a period of 24-96 weeks:

- Long-Term storage conditions: 5°C ± 3°C
- Accelerated conditions: 25°C ± 2°C
- Stress condition: 40°C ± 2°C

The program is designed for 96 weeks and data acquired during the program is analyzed by Arrhenius plots for extended shelf life calculations. The following table summarizes the sampling intervals in the program.

Table 6. Sampling intervals for the long term stability program

Study	**Storage condition**	**Sample test interval (weeks)**
Long-Term Storage Conditions	5°C ± 3°C	0,1wk, 2, 4, 6, 8, 12, 16, 20, 24, 48, 72, 96
Accelerated Conditions	25°C ± 2°C	0,1wk,2, 4, 8, 12, 16, 24, 48, 96
Stress Conditions	40°C ± 2°C	0,1wk, 2, 4, 6, 8, 12, 16, 20, 24, 48, 72, 96

Samples are tested for quality attributes using various techniques: pH measurements, total protein concentration as determined by UV-Vis, appearance (color, turbidity and visible particles), fragments including half antibody content as determined by Experion automated electrophoresis, aggregation as determined by size exclusion chromatography, sub-visible particle using Microflow Imaging (MFI), and conformational and colloidal stability. At selected time points the samples are also tested for charge variants distribution and purity by RP-LC.

Below we present data for aggregation, sub-visible particles and half-bodies content in the sample.

Size Exclusion Chromatography –is a chromatographic techniques that allows separation of monomeric forms from aggregated species present in biologics samples. The assay is performed using UPLC system equipped with Waters BEH200 SEC 1.7 um 4.6x150 mm at column temperature 30+/-0.5°C. The mobile phase used was 100 mM Sodium Phosphate buffer with 200 mM Sodium Chloride pH 6.8+/- 0.05 at flow rate of 0,3 ml/min .

The samples, prepared in duplicates, were diluted to 1 mg/ml in mobile phase and then a volume of 2 µl was injected to the system. The detection was performed by UV/Vis at 210 nm.

Table 7. SEC results for samples up to 4 weeks

% Total Aggregates by Size Exclusion Chromatography				
Time point [weeks]	**0**	**1**	**2**	**4**
F5_5°C	0.40	0.42	0.41	0.41
F5_40°C	0.40	0.45	0.47	0.56
F5_25°C	0.40	0.39	0.37	0.42
F4_5°C	0.47	0.51	0.50	0.54
F4_40°C	0.47	0.55	0.58	0.80
F4_25°C	0.47	0.49	0.48	0.52
F1_5°C	0.28	0.28	0.26	0.27
F1_40°C	0.28	0.26	0.27	0.33
F1_25°C	0.28	0.24	0.22	0.26

The data shows continuous stability of the PB006_Batch3 sample in various formulations over the period of 4 weeks at various temperatures. The long term storage and accelerated conditions show no significant increase in % aggregates indicating stability for both formulations, F1 and F4 over the time of 4 weeks.

Microflow imaging MFI is a technique that allows to characterize sub-visible particles in the range of 2µm-200µm present in drug product during its development.

Before sample preparation, the system was primed with related formulation buffer to optimize illumination first. Samples were prepared by gently mixing in circular motions after removal from storage condition. Then the sample preparation was performed under laminal flow hood. The vials were opened and 1 ml of sample was pipetted on a sample plate. The sample were prepared in duplicates. Then the plate was placed in the instrument autosampler. The samples tested were neat at target concentration of about 20 mg/ml. Table 8 shows the results for formulations F1, F4, and F5.

Table 8. MFI data for sub-visibles particles greater than 10 µ for samples up to 4 weeks in the stability program.

Sub-visible particle count by MFI				
Time point [weeks]	**0**	**1**	**2**	**4**
F5_5°C	59	13	101	74
F5_40°C	59	7	6	4
F5_25°C	59	179	640	125
F4_5°C	202	24	14	80
F4_40°C	202	2	40	616
F4_25°C	202	807	154	765
F1_5°C	187	3	286	248

F1_40°C	187	8	22	17
F1_25°C	187	25	338	235

The data shows constant stability of the PB006_Batch3 sample in three different formulations over the period of 4 weeks at 5°C, 26°C and 40°C. The long term storage and accelerated conditions show great stability for both formulations, F1 and F4 over the time of 4 weeks.

The Experion® automated electrophoresis system (Biorad) employs LabChip microfluidic technology to automate protein electrophoresis. Separation of sample takes place on the microfluidic chip. Each chip contains a series of plastic wells bonded over a small glass plate. The glass plate is etched with a network of microchannels that connects with the base of the plastic wells. The microchannels are primed, or filled with a mixture of gel matrix and stain, before the samples are applied to the wells. Once a prepared chip is placed in the electrophoresis station and the lid is closed, 16 electrode pins contact the solutions in each well. The electrophoresis station directs the samples through the microchannels by controlling the voltages and currents that are applied. The system has sensitivity comparable to that of colloidal Coomassie Blue gel staining.

The samples were prepared by 20 fold dilution in water and in accordance with the procedure and sample preparation kit provided by the manufacturer.

Table 9. Data on half bodies content by chip electroforesis using Experion.

% half antibodies by chip electrophoresis (Experion®; BioRad)				
Time point [weeks]	**0**	**1**	**2**	**4**
F5_5°C	1.53	1.93	1.75	1.685
F5_40°C	1.53	1.46	1.34	2.24
F5_25°C	1.53	1.49	1.19	1.57
F4_5°C	1.66	1.61	1.33	1.56
F4_40°C	1.66	1.72	1.23	2.025
F4_25°C	1.66	1.36	1.51	1.77
F1_5°C	1.730	0.985	1.080	1.49
F1_40°C	1.730	1.485	1.810	2.895
F1_25°C	1.730	1.445	1.180	1.66

The preliminary data for the stability program obtained using chip electrophoresis under non-reduced conditions indicates product stability in all three formulations. The level of half bodies is stable at both accelerated and long term storage conditions in all tested formulations.

The stored samples are further examined in accordance with the following table.

Table 10. Quality attributes and timepoints tested during the stability program.

Quality attribute	Timepoints (weeks)												
	0	1	2	4	6	8	12	16	20	24	48	72	96
Appearance (colour, visible particles and clarity) Visual	X	X	X	X	X	X	X	X	X	X	X	X	X
pH Potentiometric	X	X	X	X	X	X	X	X	X	X	X	X	X
Sub-visible particles Light obscuration	X	X	X	X	X	X	X	X	X	X	X	X	X
Sub-visible particles Micro-flow imaging	X	X	X	X	X	X	X	X	X	X	X	X	X
Amino acid sequence UV-LC	X	-	-	-	-	-	-	-	-	-	-	-	-
Protein concentration/Total Protein UV 280	X	X	X	X	X	X	X	X	X	X	X	X	X
N-Glycosylation Site & Antibody fragments CE reduced	X	-	-	-	-	-	-	-	-	-	-	-	-
Half antibody content & Antibody fragments CE non-reduced	X	X	X	X	X	X	X	X	X	X	X	X	X
Charge variants IEX-LC	X	X	X	X	X	X	X	X	X	X	X	X	X
Aggregation SEC-LC	X	X	X	X	X	X	X	X	X	X	X	X	X
Purity (imp.) RP-LC	X	X	X	X	X	X	X	X	X	X	X	X	X
Container Closure Integrity Dye ingress test	X	X	X	X	X	X	X	X	X	X	X	X	X
Binding to α4-integrin Direct ELISA	X	-	-	X	-	X	X	X	X	X	X	X	X

Size Exclusion Chromatography is a chromatographic technique that allows separation of monomeric forms from aggregated species present in biologics samples. The assay is performed using UPLC system equipped with Waters BEH200 SEC 1.7 um 4.6x150 mm at column temperature 30+/-0.5°C. The mobile phase is 100 mM Sodium Phosphate buffer with 200 mM Sodium Chloride pH 6.8+/- 0.05 at flow rate of 0,3 ml/min. The samples, prepared in duplicates, are diluted to 1 mg/ml in mobile phase and then a volume of 2 µl is injected to the system. The detection is performed by UV/Vis at 210 nm.

Monoclonal antibodies may display considerable heterogeneity that can be characterized by ion exchange liquid chromatography (IEX-LC). In a preferred embodiment, the separation is carried out by Cation Exchange (CEX) Ultra Performance Liquid Chromatography on UPLC H-Class Bio System using UV detection under EmpowerTM Software control. Samples are analyzed using Protein-Pak Hi Res SP (7 µm, 4.6 mm i.d. × 100 mm) in a linear gradient of NaCl. Eluents are: buffer A (14mM NaPi buffer pH 6.0) and buffer B (10.5mM NaPi buffer pH 6.0, 0.125 M NaCl). Gradient starts with pre-

equilibration of 100% buffer A in 2 min. Elution gradient starts from 10% to 30% of buffer B in 25 min, followed by a second elution step for 5 min at 30% to 60% B and re-equilibration with 90% solvent A. The total run time is 40 min. The flow rate is 0.7 ml/min. The column temperature is 40°C and elution is monitored at 220 nm. For data evaluation Waters Empower 3 software is used. The peak assignment is performed by retention time. The sample composition is determined by detecting peaks based on their retention time and the relative proportions of each peak are calculated from the peak areas. The final results are presented as a sum of acidic species, main peak and sum of basic species.

RP-LC for antibody analysis is a chromatographic method used to detect and quantify alterations in hydrophobicity of protein fragments. Briefly, before analysis monoclonal antibody sample is digested with IdeS enzyme in order to obtain Fab and Fc fragments. Retention mechanism is based on subsequent adsorption of those fragments to stationary phase and desorption after adequate elution strength is reached. Any modifications affecting hydrophobicity, will result in Fc or Fab retention time alteration. The analyzed samples are expressed as chromatogram in which populations of different hydrophobicity are divided into peaks. Hence, RP analysis of antibody fragments method provides information concerning percentage of fragments bearing hydrophobicity-altering modifications such as methionine or tryptophan oxidation. The separation is carried out by UPLC H-Class Bio System using UV detection under EmpowerTM Software control. Samples are analyzed using BEH300 C4 (1.7 µm, 2.1 mm i.d. × 100 mm) with linear gradient of 30 → 80% B for 15 minutes at flow rate of 0.2 mL/min. Mobile phases are A: 0.1% TFA in Water, B: 0.1% TFA in Acetonitrile. The column temperature is 80°C and elution is monitored at 220 nm. For data evaluation Waters Empower 3 software is used. The peak assignment is performed by retention time. The sample composition is determined by detecting peaks based on their retention time and the relative proportions of each peak are calculated from the peak areas. The final results are presented as a sum of Fc Pre Peaks, Fc Main Peak, Fc Post Peaks, $F(ab')_2$ Pre Peaks, $F(ab')_2$ main peak and $F(ab')_2$ Post Peaks. A sum of Fc Main Peak and $F(ab')_2$ main peak is termed as 'Purity'.

Non-reduced capillary electrophoresis (Non-red CE) is a electrophoretic method used to detect and quantify size heterogeneity or fragmentation of protein. Briefly, it involves molecule separation, based on their molecular size. The proteins form complexes with sodium dodecylsulphate that mask their native charge, so that the resulting charge is directly proportional to their molecular weight. When electric field is applied, the protein molecules are separated according to their mass. In a preferred embodiment, the separation is carried out in 30.2 cm, 50 µm ID, bare fused silica capillary and is performed for 28 min at 7.5 kV, with detection at 214 nm. The analyzed samples are expressed as electropherogram in which different molecular size populations are divided into peaks.

UV spectrophotometry (A280) is a technique based on Lambert-Beer law and is used to determine concentration of protein in solution. Spectra from 240 to 340 nm are recorded for samples diluted to about 0.5 ml/mL with PBS buffer. Protein concentration is

determined using absorbance at 280 nm value diminished by absorbance at 320 nm value.

Light obscuration is a compendial method for sub-visible particle analysis. The method is based on passing the liquid sample between laser source and light-sensitive detector. Particles present in the sample block certain amount of light from a laser beam and generate 'shadow'. Area of the shadow is converted into equivalent circular diameter of the particle. In a preferred embodiment, a sample is diluted three-fold with appropriate formulation buffer and analyzed in four replicates. The results are expressed as particle count for particles equal to or greater than 10 µm and particles to or greater than 25 µm.

Visual inspection is a compendial method for color, turbidity and particle content analysis. The method is based on European Pharmacopoeia chapters 2.9.20, 2.2.1 and 2.2.2. In a preferred embodiment Reference solutions Y are used as color standards, NTU3, NTU6, NTU18, NTU30, NTU60, NTU120, NTU200, NTU1000 are used as turbidity standards.

The results determined at time points 0, and after 3, 6, and 12 months of storage at 5°C are shown in the following Tables 11-13.

Table 11. Stability data for 20 mg/ml humanized IgG_4 (natalizumab) in formulation F1 stored at 5°C for 12 months.

Test (Method Number)	Reportable value / Expected Range	Timepoint			
		Start	3 months	6 months	12 months
Colour (Ph. Eur. 2.2.2)	Not more colored than Y5, B5 or BY5	complies[1]	complies	complies	complies
Visible particles (Ph. Eur. 2.9.20)	Practically free from visible particles	complies	complies	complies	complies
Clarity (Ph. Eur. 2.2.1)	NMT reference suspension III or ≤18 NTU	complies[2]	complies	complies	complies
pH (Ph. Eur. 2.2.3)	5.7 ± 0.2	5.7	5.6	5.7	5.7
Light obscuration (USP <787>)	≤ 6000 per container for particles ≥10 µm	20	10	80	75
	≤ 600 per container for particles ≥25 µm	0	0	5	40
Micro-flow imaging	1 µm ≤ x < 2 µm	13217	12513	6773	27904
	2 µm ≤ x < 10 µm	4144	5741	2422	7710
	10 µm ≤ x < 25 µm	66	254	91	98
	25 µm ≤ x < 100 µm	0	0	25	25
UV280[c]	18.0 - 22.0 mg/ml	21.1	21.2	21.1	21.4
CE reduced	Purity	99.3	-	-	-

	Sum of low molecular weight variants	0.1	-	-	-
CE non-reduced	Purity: ≥88.0%	98.0	98.1	97.9	97.6
	Sum of low molecular weights / %	1.7	1.7	1.9	1.8
	Sum of high molecular weights / %	0.2	0.2	0.2	0.6
IEX-LC	Sum of Acidic Peaks: ≤25.0%	14.8	14.5	14.4	14.6
	Sum of Basic Peaks	8.6	9.2	8.6	10.8
	Main Peak: ≥63.0%	76.5	76.3	77.0	74.6
SEC-LC	Dimers: ≤1.5%	0.5	0.5	0.6	0.7
	Higher order aggregates: ≤0.2%	0.0	0.0	0.0	0.0
	Aggregates[3]	0.5	0.5	0.6	0.7
	Main Peak: ≥95.0%	99.5	99.4	99.3	99.2
RP-LC	Purity	75.8	78.2	76.5	72.8
	Sum of oxidized species[a]	0.0	0.0	0.1	0.1
Binding to α4-integrin (	80-125%	not tested	-	101.50	105.40

- not to be analyzed
[1] Not more colored than Y5
[2] Clear to slightly opalescent (≤18 NTU)
[3] Values are sums of dimers and higher order aggregates after rounding

Table 12. Stability data for 20 mg/ml humanized IgG_4 (natalizumab) in formulation F4 stored at 5°C for 12 months.

Test (Method Number)	**Reportable value / Expected Range**	**Timepoint**			
		Start	**3 months**	**6 months**	**12 months**
Colour (Ph. Eur. 2.2.2)	Not more colored than Y5, B5 or BY5	complies[1]	complies	complies	complies
Visible particles (Ph. Eur. 2.9.20)	Practically free from visible particles	complies	complies	complies	complies
Clarity (Ph. Eur. 2.2.1)	NMT reference suspension III or ≤18 NTU	complies[2]	complies	complies	complies
pH (Ph. Eur. 2.2.3)	6.5 ± 0.2	6.6	6.4	6.5	6.5
Light obscuration (USP <787>)	≤ 6000 per container for particles ≥10 µm	5	15	70	23

	≤ 600 per container for particles ≥25 µm	0	0	30	0
Micro-flow imaging	1 µm ≤ x < 2 µm	7789	18816	15710	43166
	2 µm ≤ x < 10 µm	2916	6611	6268	12961
	10 µm ≤ x < 25 µm	74	172	205	74
	25 µm ≤ x < 100 µm	0	33	8	16
UV280	18.0 - 22.0 mg/ml	22.2	22.2	22.1	22.5
CE reduced	Purity	99.2	-	-	-
	Sum of low molecular weight variants	0.1	-	-	-
CE non-reduced	Purity: ≥88.0%	98.0	97.9	97.6	97.4
	Sum of low molecular weights / %	1.7	1.8	2.0	1.7
	Sum of high molecular weights / %	0.3	0.3	0.5	0.8
IEX-LC	Sum of Acidic Peaks: ≤25.0%	15.5	15.8	15.9	16.6
	Sum of Basic Peaks[a]	8.8	9.1	8.8	10.2
	Main Peak: ≥63.0%	75.7	75.0	75.3	73.3
SEC-LC	Dimers: ≤1.5%	1.1	1.4	1.5	1.6
	Higher order aggregates: ≤0.2%	0.0	0.0	0.1	0.1
	Aggregates[3]	1.1	1.4	1.6	1.7
	Main Peak: ≥95.0%	98.8	98.4	98.4	98.3
RP-LC (	Purity	75.4	77.8	76.7	72.8
	Sum of oxidized species	0.1	0.0	0.1	0.1
Binding to α4-integrin	80-125%	not tested	-	106.00	103.25

- not to be analyzed
[1] Not more colored than Y5
[2] Clear to slightly opalescent (≤18 NTU)
[3] Values are sums of dimers and higher order aggregates after rounding

Table 13. Stability data for 20 mg/ml humanized IgG_4 (natalizumab) in formulation F5 stored at 5°C for 12 months.

Test (Method Number)	Reportable value / Expected Range	Timepoint			
		Start	3 months	6 months	12 months
Colour (Ph. Eur. 2.2.2)	Not more colored than Y5, B5 or BY5	complies[1]	complies	complies	complies
Visible particles (Ph. Eur. 2.9.20)	Practically free from visible particles	complies	complies	complies	complies
Clarity (Ph. Eur. 2.2.1)	NMT reference suspension III or ≤18 NTU	complies[2]	complies	complies	complies
pH (Ph. Eur. 2.2.3)	6.1 ± 0.2	6.3	6.2	6.2	6.3
Light obscuration (USP <787>)	≤ 6000 per container for particles ≥10 µm	20	65	45	50
	≤ 600 per container for particles ≥25 µm	0	0	10	20
Micro-flow imaging	1 µm ≤ x < 2 µm	11506	35557	10060	28101
	2 µm ≤ x < 10 µm	5888	12128	4269	8916
	10 µm ≤ x < 25 µm	311	131	139	156
	25 µm ≤ x < 100 µm	25	8	16	33
UV280	18.0 - 22.0 mg/ml	20.5	20.5	20.8	21.1
CE reduced	Purity	99.3	-	-	-
	Sum of low molecular weight variants	0.1	-	-	-
CE non-reduced	Purity: ≥88.0%	97.8	97.9	97.7	97.4
	Sum of low molecular weights / %	1.7	1.8	1.9	1.8
	Sum of high molecular weights / %	0.5	0.3	0.4	0.8
IEX-LC	Sum of Acidic Peaks: ≤25.0%	15.1	15.1	15.1	15.4
	Sum of Basic Peaks[a]	9.0	8.9	9.0	10.3
	Main Peak: ≥63.0%	75.9	76.1	75.9	74.3
SEC-LC	Dimers: ≤1.5%	1.0	1.1	1.2	1.3
	Higher order aggregates: ≤0.2%	0.0	0.0	0.0	0.0
	Aggregates[3]				1.3
	Main Peak: ≥95.0%	99.0	98.8	98.7	98.6

RP-LC	Purity	75.8	78.2	77.4	72.4
	Sum of oxidized species	0.0	0.0	0.1	0.1
Binding to α4-integrin	80-125%	92.00	-	97.40	101.30

- not to be analyzed
[1] Not more colored than Y5
[2] Clear to slightly opalescent (≤18 NTU)
[3] Values are sums of dimers and higher order aggregates after rounding

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The results determined at time points 0, and after 3, and 6 months of storage at 25°C are shown in the following Tables 14-16.

Table 14. Stability data for 20 mg/ml humanized IgG_4 (natalizumab) in formulation F1 stored at 25°C for 6 months.

Test (Method Number)	**Reportable value / Expected Range**	**Timepoint**		
		Start	**3 months**	**6 months**
Colour (Ph. Eur. 2.2.2)	Not more colored than Y5, B5 or BY5	complies[1]	complies	complies
Visible particles (Ph. Eur. 2.9.20)	Practically free from visible particles	complies	complies	complies
Clarity (Ph. Eur. 2.2.1)	NMT reference suspension III or ≤18 NTU	complies[2]	complies	complies
pH (Ph. Eur. 2.2.3)	5.7 ± 0.2	5.7	5.7	5.6
Light obscuration (USP <787>)	≤ 6000 per container for particles ≥10 µm	20	10	35
	≤ 600 per container for particles ≥25 µm	0	0	5
Micro-flow imaging	1 µm ≤ x < 2 µm	13217	27166	27800
	2 µm ≤ x < 10 µm	4144	2761	3457
	10 µm ≤ x < 25 µm	66	74	115
	25 µm ≤ x < 100 µm	0	0	57
UV280	18.0 - 22.0 mg/ml	21.1	21.2	21.2
CE reduced	Purity	99.3	-	97.3
	Sum of low molecular weight variants	0.1	-	1.6
CE non-reduced	Purity: ≥88.0%	98.0	97.2	96.2
	Sum of low molecular weights / %	1.7	2.5	3.4

	Sum of high molecular weights / %	0.2	0.2	0.4
IEX-LC	Sum of Acidic Peaks: ≤25.0%	14.8	16.4	19.4
	Sum of Basic Peaks[a]	8.6	10.3	10.9
	Main Peak: ≥63.0%	76.5	73.3	69.7
SEC-LC	Dimers: ≤1.5%	0.5	0.7	0.6
	Higher order aggregates: ≤0.2%	0.0	0.0	0.0
	Aggregates[3]	0.5	0.7	0.6
	Main Peak: ≥95.0%	99.5	99.2	95.5
RP-LC	Purity	75.8	78.1	76.5
	Sum of oxidized species	0.0	0.1	0.6
Binding to α4-integrin	80-125%	Not tested	104.00	104.00

- not to be analyzed
[1] Not more colored than Y5
[2] Clear to slightly opalescent (≤18 NTU)
[3] Values are sums of dimers and higher order aggregates after rounding

Table 15. Stability data for 20 mg/ml humanized IgG_4 (natalizumab) in formulation F4 stored at 25°C for 6 months.

Test (Method Number)	**Reportable value / Expected Range**	**Timepoint**		
		Start	**3 months**	**6 months**
Colour (Ph. Eur. 2.2.2)	Not more colored than Y5, B5 or BY5	complies[1]	complies	complies
Visible particles (Ph. Eur. 2.9.20)	Practically free from visible particles	complies	complies	complies
Clarity (Ph. Eur. 2.2.1)	NMT reference suspension III or ≤18 NTU	complies[2]	complies	complies
pH (Ph. Eur. 2.2.3)	6.5 ± 0.2	6.6	6.4	6.4
Light obscuration (USP <787>)	≤ 6000 per container for particles ≥10 µm	5	15	20
	≤ 600 per container for particles ≥25 µm	0	0	0
Micro-flow imaging	1 µm ≤ x < 2 µm	7789	23430	14584
	2 µm ≤ x < 10 µm	2916	8856	4785

	10 µm ≤ x < 25 µm	74	107	156
	25 µm ≤ x < 100 µm	0	25	33
UV280	18.0 - 22.0 mg/ml	22.2	22.1	22.1
CE reduced	Purity	99.2	-	95.1
	Sum of low molecular weight variants	0.1	-	3.1
CE non-reduced	Purity: ≥88.0%	98.0	96.5	95.1
	Sum of low molecular weights / %	1.7	2.9	3.7
	Sum of high molecular weights / %	0.3	0.6	1.2
IEX-LC	Sum of Acidic Peaks: ≤25.0%	15.5	23.6	30.4
	Sum of Basic Peaks	8.8	9.5	9.0
	Main Peak: ≥63.0%	75.7	66.9	60.5
SEC-LC	Dimers: ≤1.5%	1.1	1.6	1.9
	Higher order aggregates: ≤0.2%	0.0	0.1	0.1
	Aggregates[3]	1.1	1.7	2.0
	Main Peak: ≥95.0%	98.8	98.2	97.9
RP-LC	Purity	75.4	77.2	73.6
	Sum of oxidized species	0.1	0.2	0.4
Binding to α4-integrin	80-125%	Not tested	110.50	100.25

- not to be analyzed
[1] Not more colored than Y5
[2] Clear to slightly opalescent (≤18 NTU)
[3] Values are sums of dimers and higher order aggregates after rounding

Table 16. Stability data for 20 mg/ml humanized IgG_4 (natalizumab) in formulation F5 stored at 25°C for 6 months.

Test (Method Number)	**Reportable value / Expected Range**	**Timepoint**		
		Start	**3 months**	**6 months**
CE reduced	Purity	99.3	-	96.3

	Sum of low molecular weight variants	0.1	-	2.4
CE non-reduced	Purity: ≥88.0%	97.8	97.1	96.2
	Sum of low molecular weights / %	1.7	2.5	3.3
	Sum of high molecular weights / %	0.5	0.4	0.5
IEX-LC	Sum of Acidic Peaks: ≤25.0%	15.1	19.2	23.4
	Sum of Basic Peaks	9.0	9.3	9.0
	Main Peak: ≥63.0%	75.9	71.5	67.6
SEC-LC	Dimers: ≤1.5%	1.0	1.2	1.3
	Higher order aggregates: ≤0.2%	0.0	0.0	0.1
	Aggregates[3]	1.0	1.2	1.4
	Main Peak: ≥95.0%	99.0	98.7	98.4
RP-LC	Purity	75.8	78.3	77.0
	Sum of oxidized species	0.0	0.2	0.3
Binding to α4-integrin	80-125%	Not tested	103.50	102.50

- not to be analyzed
[1] Not more colored than Y5
[2] Clear to slightly opalescent (≤18 NTU)
[3] Values are sums of dimers and higher order aggregates after rounding

The results determined at time points 0, and after 3 months of storage at 40 °C are shown in the following Tables 17-19.

Table 17. Stability data for 20 mg/ml humanized IgG_4 (natalizumab) in formulation F1 stored at 40°C for 3 months.

Test (Method Number)	**Reportable value / Expected Range**	**Timepoint**	
		Start	**3 months**
Colour (Ph. Eur. 2.2.2)	Not more colored than Y5, B5 or BY5	complies[1]	complies
Visible particles (Ph. Eur. 2.9.20)	Practically free from visible particles	complies	complies
Clarity (Ph. Eur. 2.2.1)	NMT reference suspension III or ≤18 NTU	complies[2]	complies

pH (Ph. Eur. 2.2.3)	5.7 ± 0.2	5.7	5.7
Light obscuration (USP <787>)	≤ 6000 per container for particles ≥10 µm	20	10
	≤ 600 per container for particles ≥25 µm	0	0
Micro-flow imaging	1 µm ≤ x < 2 µm	13217	52831
	2 µm ≤ x < 10 µm	4144	19071
	10 µm ≤ x < 25 µm	66	426
	25 µm ≤ x < 100 µm	0	8
UV280	18.0 - 22.0 mg/ml	21.1	21.6
CE reduced	Purity	99.3	94.9
	Sum of low molecular weight variants	0.1	3.6
CE non-reduced	Purity: ≥88.0%	98,0	90.5
	Sum of low molecular weights / %	1.7	9.1
	Sum of high molecular weights / %	0.2	0.4
IEX-LC	Sum of Acidic Peaks: ≤25.0%	14.8	36.1
	Sum of Basic Peaks	8.6	13.9
	Main Peak: ≥63.0%	76.5	50.0
SEC-LC	Dimers: ≤1.5%	0.5	1.3[4]
	Higher order aggregates: ≤0.2%	0,0	0.75[5]
	Aggregates[3]	0.5	2.0
	Main Peak: ≥95.0%	99.5	95.3[6]
RP-LC	Purity	75.8	77.7
	Sum of oxidized species	0,0	0.8
Binding to α4-integrin	80-125%	Not tested	101.55

- not to be analyzed
[1] Not more colored than Y5
[2] Clear to slightly opalescent (≤18 NTU)
[3] Values are sums of dimers and higher order aggregates after rounding
[4] Reanalysis resulted in a value of 1.4
[5] Reanalysis resulted in a value of 0.7

[6] Reanalysis resulted in a value of 95.0

Table 18. Stability data for 20 mg/ml humanized IgG_4 (natalizumab) in formulation F4 stored at 40°C for 3 months.

Test (Method Number)	Reportable value / Expected Range	Timepoint	
		Start	3 months
Colour (Ph. Eur. 2.2.2)	Not more colored than Y5, B5 or BY5	complies[1]	complies
Visible particles (Ph. Eur. 2.9.20)	Practically free from visible particles	complies	complies
Clarity (Ph. Eur. 2.2.1)	NMT reference suspension III or ≤18 NTU	complies[2]	complies
pH (Ph. Eur. 2.2.3)	6.5 ± 0.2	6.6	6.4
Light obscuration (USP <787>)	≤ 6000 per container for particles ≥10 µm	5	30
	≤ 600 per container for particles ≥25 µm	0	0
Micro-flow imaging	1 µm ≤ x < 2 µm	7789	41721
	2 µm ≤ x < 10 µm	2916	20754
	10 µm ≤ x < 25 µm	74	1695
	25 µm ≤ x < 100 µm	0	270
UV280	18.0 - 22.0 mg/ml	22.2	22.3
CE reduced	Purity	99.2	90.1
	Sum of low molecular weight variants	0.1	7.2
CE non-reduced	Purity: ≥88.0%	98,0	88.5
	Sum of low molecular weights / %	1.7	10.1
	Sum of high molecular weights / %	0.3	1.4
IEX-LC	Sum of Acidic Peaks: ≤25.0%	15.5	67.8
	Sum of Basic Peaks	8.8	9.7
	Main Peak: ≥63.0%	75.7	22.5
SEC-LC	Dimers: ≤1.5%	1.1	2.8

	Higher order aggregates: ≤0.2%	0,0	0.2
	Aggregates[3]	1.1	3.0
	Main Peak: ≥95.0%	98.8	95.1
RP-LC	Purity	75.4	71.1
	Sum of oxidized species	0.1	1.5
Binding to α4-integrin	80-125%	Not tested	100.75

- not to be analyzed
[1] Not more colored than Y5
[2] Clear to slightly opalescent (≤18 NTU)
[3] Values are sums of dimers and higher order aggregates after rounding

Table 19. Stability data for 20 mg/ml humanized IgG_4 (natalizumab) in formulation F5 stored at 40°C for 3 months.

Test (Method Number)	**Reportable value / Expected Range**	**Timepoint**	
		Start	**3 months**
Colour (Ph. Eur. 2.2.2)	Not more colored than Y5, B5 or BY5	complies[1]	complies
Visible particles (Ph. Eur. 2.9.20)	Practically free from visible particles	complies	complies
Clarity (Ph. Eur. 2.2.1)	NMT reference suspension III or ≤18 NTU	complies[2]	complies
pH (Ph. Eur. 2.2.3)	6.1 ± 0.2	6.3	6.2
Light obscuration (USP <787>)	≤ 6000 per container for particles ≥10 µm	20	130
	≤ 600 per container for particles ≥25 µm	0	0
Micro-flow imaging	1 µm ≤ x < 2 µm	11506	84008
	2 µm ≤ x < 10 µm	5888	28694
	10 µm ≤ x < 25 µm	311	1704
	25 µm ≤ x < 100 µm	25	229
UV280	18.0 - 22.0 mg/ml	20.5	20.5
CE reduced	Purity	99.3	93.3
	Sum of low molecular weight variants	0.1	5.1

CE non-reduced	Purity: ≥88.0%	97.8	90.4
	Sum of low molecular weights / %	1.7	9.1
	Sum of high molecular weights / %	0.5	0.4
IEX-LC	Sum of Acidic Peaks: ≤25.0%	15.1	48.6
	Sum of Basic Peaks	9.0	9.7
	Main Peak: ≥63.0%	75.9	41.7
SEC-LC	Dimers: ≤1.5%	1.0	1.5
	Higher order aggregates: ≤0.2%	0.0	0.1
	Aggregates[3]		
	Main Peak: ≥95.0%	99.0	96.7
RP-LC	Purity	75.8	75.4
	Sum of oxidized species	0.0	1.2
Binding to α4-integrin	80-125%	Not tested	104.50

- not to be analyzed
[1] Not more colored than Y5
[2] Clear to slightly opalescent (≤18 NTU)
[3] Values are sums of dimers and higher order aggregates after rounding

The results show that both formulation F1 and formulation F4 exhibit at least a similar stability as compared to the prior art formulation F5. Upon storage at 5°C and 40°C, formulation F1 shows slightly improved quality attributes as compared to formulation F5.

Example 4

It was further decided to test additional formulation variants of formulation F1 in order to identify formulation with improved freeze-thaw stability. The following formulations of 20 mg/ml natalizumab were tested:

Formulation F1: 10 mM L-histidine, 150 mM NaCl, 0.02% (w/v) polysorbate 80, pH 5.7.

Formulation F5: 10 mM sodium phosphate, 140 mM NaCl, 0.02% polysorbate 80, pH 6.1 (control, Tysabri formulation)

Formulation F6: 10 mM L-histidine, 150 mM NaCl, **0.2%** polysorbate 80, pH 5.7.

Formulation F7: 10 mM L-histidine, **20 mM** NaCl, 0.02% polysorbate, pH 5.7.

Formulation F8: 10 mM L-histidine, **20 mM** NaCl, **5% (w/v) sorbitol**, 0.02% polysorbate 80, pH 5.7.

The antibody for the study was produced at Polpharma Biologics (PB006_Batch3). The results are provided in terms of % average content of aggregates, as determined using size exclusion chromatography. The assay is performed using UPLC system equipped with Waters BEH200 SEC 1.7 um 4.6x150 mm at column temperature 30+/-0.5°C. The mobile phase used was 100 mM sodium phosphate buffer with 200 mM sodium chloride pH 6.8+/- 0.05 at a flow rate of 0.3 ml/min. The samples were diluted to 1 mg/ml in mobile phase and then a volume of 2 µl was injected to the system. The measurement was performed at 210 nm. The following table indicates the results for different methods of freeze-thawing.

Table 20. % Average content of aggregates for samples subjected to different types of freeze-thaw cycles, as determined using size exclusion chromatography.

	F1	F6	F7	F8	F5
Not frozen material (1 sample)	0.45	0.47	0.47	0.47	0.51
-20°C 1 week of freezing (average of 5 samples)	0.51	0.75	0.55	0.49	0.69
-80°C 1 week of freezing (average of 5 samples)	0.78	1.40	0.47	0.47	0.75
Liq. Nitrogen -> -80°C 1 week of freezing (average of 5 samples)	0.90	1.44	0.47	0.48	0.69

The data shown in the above table demonstrates that formulations F7 and F8 show a superior freeze-thaw stability as compared to formulations F1 (cf. Examples 2 and 3), F6, and F5 (control, Tysabri formulation).

Example 5

The following formulations of 20 mg/ml natalizumab were additionally tested for their ability to confer stability to humanized IgG_4 such as natalizumab:Formulation F9: 10 mM L-histidine, 150 mM NaCl, 10 mM L-methionine, 0.02% (w/v) polysorbate 80, pH 5.7.

Formulation F10: 10 mM L-histidine, 150 mM NaCl, 10 mM L-methionine, 30 mM glycine, 0.02% (w/v) polysorbate 80, pH 6.1.

Formulation F11: 30 mM sodium phosphate, 5% (w/v) sucrose, 10 mM L-arginine-HCl, 0.02% polysorbate 80, pH 6.1

Formulation F12: 30 mM sodium phosphate, 5% (w/v) sucrose, 10 mM L-arginine-HCl, 2 mM L-methionine, 0.02% polysorbate 80, pH 6.1

Formulation F13: 30 mM sodium phosphate, 5% (w/v) sorbitol, 50 mM NaCl, 0.02% polysorbate 80, pH 6.5

Formulation F14: 30 mM sodium phosphate, 5% (w/v) sorbitol, 50 mM NaCl, 0.02% polysorbate 80, pH 5.7

The antibody for the study was produced at Polpharma Biologics. The formulations have been stored at 5°C, 25°C and 40°C for 12 weeks, and analyzed by CEX, SEC, CE non-red, ELISA, LC-MS, and DLS, as described in Example 3. The changes in the various quality attributes over storage are shown in the following tables.

Table 21. Formulation F9 - changes in various quality attributes after storage for 12 weeks at 5 °C, 25 °C and 40 °C.

		Formulation F9			
Parameter (method)	Limits	0 weeks	12 weeks @ 5°C	12 weeks @ 25°C	12 weeks @ 40°C
main peak [%] (CEX)	≥ 63.00%	70.76	70.74	69.71	36.65
		70.92			
acidic species [%] (CEX)	≤ 25.00%	14.11	13.49	14.52	46.66
		14.11			
Main Peak [%] (SEC)	≥ 95.00%	98.38	98.87	98.73	94.57
		98.47			
dimers [%] (SEC)	≤ 1.50%	0.36	0.44	0.56	1.16
		0.39			
HMWI [%] (SEC)	≤ 0.20%	<0.1%	<0.1%	<0.1%	0.77
		<0.1%			
main peak [%] (CE non-red)	≥ 88.00%	97.26	98.13	96.8	88.55
		97.42			
integrin α4β1 binding [%] (ELISA)	80.00-125.00%	103.50			88.15
		103.50			
Met 255 oxidation [%] (LC-MS)		4.8	4.2	4.2	5.0
		5.0			
hydrodynamic radius [nm] (DLS)		6.1	6.2	6.1	6.3
		6.1			

Table 22. Formulation F10 - changes in various quality attributes after storage for 12 weeks at 5 °C, 25 °C and 40 °C.

		Formulation F10			
Parameter (method)	Limits	0 weeks	12 weeks @ 5°C	12 weeks @ 25°C	12 weeks @ 40°C
main peak [%] (CEX)	≥ 63.00%	70.88	70.79	68.67	34.52
		70.86			
acidic species [%]	≤ 25.00%	14.18	13.79	16	51.41

(CEX)		14.22			
Main Peak [%] (SEC)	≥ 95.00%	98.45	98.87	98.71	95.36
		98.46			
dimers [%] (SEC)	≤ 1.50%	0.37	0.47	0.46	0.79
		0.40			
HMWI [%] (SEC)	≤ 0.20%	N/D	N/D	N/D	0.38
		<0.1%			
main peak [%] (CE non-red)	≥ 88.00%	97.39	97.73	96.96	86.8
		97.23			
integrin α4β1 binding [%] (ELISA)	80.00-125.00%	102.00			85.85
		102.00			
Met 255 oxidation [%] (LC-MS)		4.9	4.2	4.0	4.5
		4.6			
hydrodynamic radius [nm] (DLS)		6.1	6.2	6.1	6.1
		6.1			

Table 23. Formulation F11 - changes in various quality attributes after storage for 12 weeks at 5 °C, 25 °C and 40 °C.

		Formulation F11			
Parameter (method)	Limits	0 weeks	12 weeks @ 5°C	12 weeks @ 25°C	12 weeks @ 40°C
main peak [%] (CEX)	≥ 63.00%	70.92	70.38	55.76	25.29
		70.92			23.97
acidic species [%] (CEX)	≤ 25.00%	14.20	13.80	29.4	61.1
		14.05			67.67
Main Peak [%] (SEC)	≥ 95.00%	98.47	98.78	98.36	94.03
		98.42			59.84
dimers [%] (SEC)	≤ 1.50%	0.40	0.60	0.84	2.29
		0.46			5.99
HMWI [%] (SEC)	≤ 0.20%	<0.1%	<0.1%	<0.1%	0.70
		<0.1%			10.05
main peak [%] (CE non-red)	≥ 88.00%	97.29	98.08	96.67	88.75
		97.12			62.07
integrin α4β1 binding [%] (ELISA)	80.00-125.00%	100.60			87.80
		100.60			33.25
Met 255 oxidation [%] (LC-MS)		4.9	4.8	5.0	11.63
		4.9			13.24
hydrodynamic radius [nm] (DLS)		11.9	9.5	29.0	7.40
		11.9			24.27

Table 24. Formulation F12 - changes in various quality attributes after storage for 12 weeks at 5 °C, 25 °C and 40 °C.

		Formulation F12			
Parameter (method)	Limits	0 weeks	12 weeks @ 5°C	12 weeks @ 25°C	12 weeks @ 40°C
main peak [%] (CEX)	≥ 63.00%	70.86	70.5	65.64	27.98
		70.87			
acidic species [%] (CEX)	≤ 25.00%	14.1	13.83	18.08	58.66
		14.3			
Main Peak [%] (SEC)	≥ 95.00%	98.40	98.69	98.48	95.38
		98.39			
dimers [%] (SEC)	≤ 1.50%	0.45	0.66	0.75	1.47
		0.50			
HMWI [%] (SEC)	≤ 0.20%	<0.1%	<0.1%	<0.1%	0.42
		<0.1%			
main peak [%] (CE non-red)	≥ 88.00%	97.39	98.01	96.86	88.59
		97.32			
integrin α4β1 binding [%] (ELISA)	80.00-125.00%	92.15			90.45
		92.15			
Met 255 oxidation [%] (LC-MS)		4.8	4.4	4.4	5.7
		4.9			
hydrodynamic radius [nm] (DLS)		7.2	7.3	7.3	7.7
		7.2			

Table 25. Formulation F13 - changes in various quality attributes after storage for 12 weeks at 5 °C, 25 °C and 40 °C.

		Formulation F13			
Parameter (method)	Limits	0 weeks	12 weeks @ 5°C	12 weeks @ 25°C	12 weeks @ 40°C
main peak [%] (CEX)	≥ 63.00%	70.87	70.34	59.44	16.85
acidic species [%] (CEX)	≤ 25.00%	14.09	13.77	24.35	71.06
Main Peak [%] (SEC)	≥ 95.00%	98.31	98.32	97.92	92.56
dimers [%] (SEC)	≤ 1.50%	0.60	1.02	1.32	3.45
HMWI [%] (SEC)	≤ 0.20%	<0.1%	<0.1%	<0.1%	0.78
main peak [%] (CE non-red)	≥ 88.00%	97.34	98.04	96.6	86.46
integrin α4β1 binding [%] (ELISA)	80.00-125.00%	103.50			87.80
		103.50			

Met 255 oxidation [%] (LC-MS)		4.5	4.5	5.6	11.6
		4.5			
hydrodynamic radius [nm] (DLS)		7.0	7.1	7.2	7.3
		7.0			

Table 26. Formulation F14 - changes in various quality attributes after storage for 12 weeks at 5 °C, 25 °C and 40 °C.

		Formulation F14			
Parameter (method)	Limits	0 weeks	12 weeks @ 5°C	12 weeks @ 25°C	12 weeks @ 40°C
main peak [%] (CEX)	≥ 63.00%	70.89	70.57	68.21	28.34
		70.87			
acidic species [%] (CEX)	≤ 25.00%	14.17	13.54	15.18	55.62
		14.15			
Main Peak [%] (SEC)	≥ 95.00%	98.38	98.70	98.35	94.61
		98.39			
dimers [%] (SEC)	≤ 1.50%	0.43	0.67	0.77	2.12
		0.45			
HMWI [%] (SEC)	≤ 0.20%	<0.1%	<0.1%	<0.1%	0.58
		<0.1%			
main peak [%] (CE non-red)	≥ 88.00%	97.3	98.13	97.13	89.37
		97.21			
integrin α4β1 binding [%] (ELISA)	80.00-125.00%	101.00			87.95
		101.00			
Met 255 oxidation [%] (LC-MS)		4.5	4.7	4.7	7.0
		4.9			
hydrodynamic radius [nm] (DLS)		7.0	7.1	7.3	7.2
		7.0			

As a result, the histidine-based formulations F9 and F10 demonstrated an advantage over phosphate-based formulations and in certain parameters such as chemical and colloidal stability (% dimer and % acidic species) also over the prior art formulation F5 (cf. Tables 13, 16, and 19 above). While formulations F9 and F10 are generally comparable, formulation F9 is chemically more stable (lower percentage of acidic species variants), while formulation F10 is more colloidally stable (lower percentage of dimers and HMWI). Among the sodium phosphate based formulations, formulations F14 and F12 presented the most favorable performance. Formulation F11 demonstrated advantage over formulation F13. Formulations F11, F12. F13, and F14 often showed a comparable performance if stored for 3 months at 5°C or 25°C as compared to formulation F5 (cf. Tables 13, 16, and 19 above). As compared to the histidine-based formulations F9 and F10, the phosphate-based formulations F11, F12, F13, and F14 showed an increased dimer formation at 5°C, 25°C, and 40°C. In addition, formulations F11 and F13 show a high level of acidic variants (F11 at 25°C; and F13 at 40°C), and high methionine 225

oxidation levels (above 10%). Formulation F11 also exhibited a change in sample color and hydrodynamic radius with multimodal size distribution.

Example 6

In light of the results of Example 5, the following formulations of 20 mg/ml natalizumab, which represent variants of formulations F1, F9 and F10 were additionally tested for their ability to confer stability to humanized IgG_4 such as natalizumab:

Table 27. Composition of formulations F15-F23 comprising 20 mg/ml humanized IgG_4 (natalizumab).

F1 10 mM Histidine 150 mM NaCl 0.02% PS80 pH 5.7	**F15**	10 mM L-histidine, **2.5 mM Na-phosphate**, 150 mM NaCl, 0.02% PS80, pH 5.7
	F16	10 mM L-histidine, **2.5 mM Na-phosphate, 20 mM NaCl,** 0.02% PS80, pH 5.7
	F17	**25 mM L-histidine,** 150 mM NaCl, 0.02% PS80, pH 5.7
F9 10 mM Histidine 150 mM NaCl 10 mM Methionine 0.02% PS80 pH 5.7	**F18**	10 mM L-histidine, **2.5 mM Na-phosphate,** 150 mM NaCl, 10 mM L-methionine, 0.02% PS80, pH 5.7
	F19	10 mM L-histidine, **2.5 mM Na-phosphate, 20 mM NaCl,** 10 mM L-methionine, 0.02% PS80, pH 5.7
	F20	**25 mM L-histidine,** 150 mM NaCl, 10 mM L-methionine, 0.02% PS80, pH 5.7
F10 10 mM Histidine 150 mM NaCl 10 mM Methionine 30 mM Glycine 0.02% PS80 pH 6.1	**F21**	10 mM L-histidine, **2.5 mM Na-phosphate,** 150 mM NaCl, 10 mM Methionine, 30 mM Glycine, 0.02% PS80, pH 6.1
	F22	10 mM L-histidine, **2.5 mM Na-phosphate, 20 mM NaCl,** 10 mM Methionine, 30 mM Glycine, 0.02% PS80, pH 6.1
	F23	**25 mM L-histidine,** 150 mM NaCl, 10 mM Methionine, 30 mM Glycine, 0.02% PS80, pH 6.1

The antibody for the study was produced at Polpharma Biologics. The formulations have been subjected to three freeze-thaw cycles (freezing for 22 hours at -80°C; thawing for 2 hours at room temperature), or stored at 45°C for up to 14 days (samples taken at day 3, 7 and 14). The samples were analyzed by CEX, SEC, CE non-red, RP, A280, and visual inspection, as described in Example 3.

Size Exclusion Chromatography is a chromatographic technique that allows separation of monomeric forms from aggregated species present in biologics samples. The assay is performed using UPLC system equipped with Waters BEH200 SEC 1.7 um 4.6x150 mm at column temperature 30+/-0.5°C. The mobile phase is 100 mM Sodium Phosphate buffer with 200 mM Sodium Chloride pH 6.8+/- 0.05 at flow rate of 0,3 ml/min. The samples, prepared in duplicates, are diluted to 1 mg/ml in mobile phase and then a volume of 2 µl is injected to the system. The detection is performed by UV/Vis at 210 nm.

Monoclonal antibodies may display considerable heterogeneity that can be characterized by ion exchange liquid chromatography (IEX-LC). In a preferred embodiment, the

separation is carried out by Cation Exchange (CEX) Ultra Performance Liquid Chromatography on UPLC H-Class Bio System using UV detection under EmpowerTM Software control. Samples are analyzed using Protein-Pak Hi Res SP (7 µm, 4.6 mm i.d. × 100 mm) in a linear gradient of NaCl. Eluents are: buffer A (14mM NaPi buffer pH 6.0) and buffer B (10.5mM NaPi buffer pH 6.0, 0.125 M NaCl). Gradient starts with pre-equilibration of 100% buffer A in 2 min. Elution gradient starts from 10% to 30% of buffer B in 25 min, followed by a second elution step for 5 min at 30% to 60% B and re-equilibration with 90% solvent A. The total run time is 40 min. The flow rate is 0.7 ml/min. The column temperature is 40°C and elution is monitored at 220 nm. For data evaluation Waters Empower 3 software is used. The peak assignment is performed by retention time. The sample composition is determined by detecting peaks based on their retention time and the relative proportions of each peak are calculated from the peak areas. The final results are presented as a sum of acidic species, main peak and sum of basic species.

RP-LC for antibody analysis is a chromatographic method used to detect and quantify alterations in hydrophobicity of protein fragments. Briefly, before analysis monoclonal antibody sample is digested with IdeS enzyme in order to obtain Fab and Fc fragments. Retention mechanism is based on subsequent adsorption of those fragments to stationary phase and desorption after adequate elution strength is reached. Any modifications affecting hydrophobicity, will result in Fc or Fab retention time alteration. The analyzed samples are expressed as chromatogram in which populations of different hydrophobicity are divided into peaks. Hence, RP analysis of antibody fragments method provides information concerning percentage of fragments bearing hydrophobicity-altering modifications such as methionine or tryptophan oxidation. The separation is carried out by UPLC H-Class Bio System using UV detection under EmpowerTM Software control. Samples are analyzed using BEH300 C4 (1.7 µm, 2.1 mm i.d. × 100 mm) with linear gradient of 30 → 80% B for 15 minutes at flow rate of 0.2 mL/min. Mobile phases are A: 0.1% TFA in Water, B: 0.1% TFA in Acetonitrile. The column temperature is 80°C and elution is monitored at 220 nm. For data evaluation Waters Empower 3 software is used. The peak assignment is performed by retention time. The sample composition is determined by detecting peaks based on their retention time and the relative proportions of each peak are calculated from the peak areas. The final results are presented as a sum of Fc Pre Peaks, Fc Main Peak, Fc Post Peaks, $F(ab')_2$ Pre Peaks, $F(ab')_2$ main peak and $F(ab')_2$ Post Peaks. A sum of Fc Main Peak and $F(ab')_2$ main peak is termed as 'Purity'.

Non-reduced capillary electrophoresis (Non-red CE) is a electrophoretic method used to detect and quantify size heterogeneity or fragmentation of protein. Briefly, it involves molecule separation, based on their molecular size. The proteins form complexes with sodium dodecylsulphate that mask their native charge, so that the resulting charge is directly proportional to their molecular weight. When electric field is applied, the protein molecules are separated according to their mass. In a preferred embodiment, the separation is carried out in 30.2 cm, 50 µm ID, bare fused silica capillary and is performed

for 28 min at 7.5 kV, with detection at 214 nm. The analyzed samples are expressed as electropherogram in which different molecular size populations are divided into peaks.

UV spectrophotometry (A280) is a technique based on Lambert-Beer law and is used to determine concentration of protein in solution. Spectra from 240 to 340 nm are recorded for samples diluted to about 0.5 ml/mL with PBS buffer. Protein concentration is determined using absorbance at 280 nm value diminished by absorbance at 320 nm value.

Visual inspection is a compendial method for color, turbidity and particle content analysis. The method is based on European Pharmacopoeia chapters 2.9.20, 2.2.1 and 2.2.2. In a preferred embodiment Reference solutions Y are used as color standards, NTU3, NTU6, NTU18, NTU30, NTU60, NTU120, NTU200, NTU1000 are used as turbidity standards.

All samples analyzed during Forced Degradation study were consistent with following criteria assessed by visual inspection: Practically free from visible particles(visible particles), ≤Y5 (color), ≤NTU18 (clarity). The changes in the other various quality attributes over storage are shown in the following tables.

Table 28. Change in IgG_4 concentration [mg/ml] in formulationf F1, F9, F10, F5 and F15-F23.

Formulation	T0	Freeze/Thaw	Thermal Stress
		After 3 cycles	14 days at 45°C
F1	22.08	22.20	22.24
F15	21.78	21.56	21.61
F16	21.42	21.48	21.64
F17	21.02	21.13	21.23
F9	20.43	20.27	20.55
F18	21.02	21.07	21.07
F19	21.02	21.09	21.17
F20	21.55	21.66	21.77
F10	19.94	19.91	20.25
F21	20.66	20.77	20.87
F22	19.81	19.85	19.92
F23	20.85	20.90	21.05
F5	19.53	19.48	19.69

Table 29. Change in purity (RP-LC) in formulationf F1, F9, F10, F5 and F15-F23 expressed as %.

Formulation	T0	Freeze/Thaw	Thermal Stress
		After 3 cycles	14 days at 45°C
F1	82.1	82.6	80.6
F15	81.4	82.0	80.4

F16	81.5	82.0	79.5
F17	81.6	82.0	80.3
F9	81.4	82.0	80.4
F18	81.4	82.0	80.2
F19	81.2	82.2	79.6
F20	81.9	82.1	79.8
F10	81.7	81.9	80.4
F21	81.8	81.8	80.5
F22	81.7	81.7	80.3
F23	82.0	81.8	80.3
F5	88.6	89.0	87.4

Table 30. Change in aggregate formation [%] induced by 1, 2, or 3 freeze-thaw cycles, as determined by SEC-LC in formulations F1, F9, F10, F5, and F15-F23.

	T0				Freeze/ Thaw											
					After 1 cycle				After 2 cycles				After 3 cycles			
	% Monomer	% Dimer	% HMWI	Total aggregates [%]	% Monomer	% Dimer	% HMWI	Total aggregates [%]	% Monomer	% Dimer	% HMWI	Total aggregates [%]	% Monomer	% Dimer	% HMWI	Total aggregates [%]
F1	98.10	0.37	N/D	0.37	97.12	0.56	<0.1	0.59	97.10	0.91	0.22	1.14	96.42	1.15	0.30	1.44
F15	97.93	0.37	N/D	0.40	97.17	0.51	<0.1	0.53	97.49	0.72	<0.1	0.79	96.79	0.90	0.14	1.04
F16	97.93	0.37	N/D	0.41	97.27	0.41	N/D	0.41	97.68	0.44	N/D	0.47	97.38	0.39	N/D	0.39
F17	98.05	0.36	N/D	0.37	97.23	0.49	N/D	0.49	97.57	0.54	N/D	0.56	97.14	0.64	N/D	0.64
F9	98.02	0.36	N/D	0.37	96.96	0.63	<0.1	0.68	97.21	0.88	<0.1	0.98	96.45	1.15	0.23	1.38
F18	97.93	0.37	N/D	0.40	97.17	0.42	N/D	0.42	97.56	0.57	<0.1	0.60	97.04	0.72	<0.1	0.76
F19	98.01	0.38	N/D	0.39	97.29	0.39	N/D	0.39	97.77	0.37	N/D	0.37	97.40	0.36	N/D	0.36
F20	97.94	0.37	N/D	0.40	97.24	0.37	N/D	0.37	97.68	0.47	N/D	0.47	97.30	0.47	N/D	0.47
F10	97.95	0.38	N/D	0.39	97.24	0.37	N/D	0.37	97.72	0.42	N/D	0.42	97.37	0.43	N/D	0.43
F21	97.84	0.40	N/D	0.43	97.20	0.39	N/D	0.39	97.66	0.42	N/D	0.42	97.23	0.41	N/D	0.41
F22	97.90	0.37	N/D	0.41	97.12	0.39	N/D	0.39	97.63	0.38	N/D	0.40	97.32	0.39	N/D	0.39
F23	97.92	0.36	N/D	0.38	97.23	0.36	N/D	0.36	97.70	0.37	N/D	0.37	97.39	0.40	N/D	0.40
F5	97.45	0.29	N/D	0.29	97.35	0.35	N/D	0.35	97.74	0.48	N/D	0.48	97.07	0.70	0.12	0.81

Table 31. Change in aggregate formation [%] after 3, 7, or 14 days of storage at 45°C, as determined by SEC-LC in formulations F1, F9, F10, F5, and F15-F23.

	T0				Thermal Stress											
					3 days at 45 °C				7 days at 45 °C				14 days at 45 °C			
	% Monomer	% Dimer	% HMWI	Total aggregates [%]	% Monomer	% Dimer	% HMWI	Total aggregates [%]	% Monomer	% Dimer	% HMWI	Total aggregates [%]	% Monomer	% Dimer	% HMWI	Total aggregates [%]
F1	98.10	0.37	N/D	0.37	97.04	0.35	N/D	0.35	96.71	0.36	N/D	0.36	96.06	0.61	0.33	0.93
F15	97.93	0.37	N/D	0.40	97.14	0.34	N/D	0.34	96.66	0.37	N/D	0.37	95.84	0.50	0.24	0.74
F16	97.93	0.37	N/D	0.41	96.99	0.36	N/D	0.36	96.75	0.37	N/D	0.37	96.22	0.51	0.20	0.71
F17	98.05	0.36	N/D	0.37	96.97	0.33	N/D	0.33	96.70	0.34	N/D	0.34	95.65	0.58	0.46	1.04
F9	98.02	0.36	N/D	0.37	97.03	0.33	N/D	0.33	96.41	0.33	N/D	0.33	96.03	0.48	0.34	0.83
F18	97.93	0.37	N/D	0.40	97.02	0.34	N/D	0.34	96.37	0.34	N/D	0.34	96.08	0.47	0.24	0.71
F19	98.01	0.38	N/D	0.39	97.01	0.33	N/D	0.33	96.63	0.34	N/D	0.34	95.93	0.45	0.20	0.65
F20	97.94	0.37	N/D	0.40	97.03	0.31	N/D	0.31	96.64	0.32	N/D	0.32	95.55	0.49	0.52	1.01
F10	97.95	0.38	N/D	0.39	96.97	0.32	N/D	0.32	96.62	0.32	N/D	0.32	96.29	0.35	N/D	0.35
F21	97.84	0.40	N/D	0.43	96.97	0.32	N/D	0.32	96.49	0.34	N/D	0.34	96.27	0.35	N/D	0.35
F22	97.90	0.37	N/D	0.41	96.92	0.34	N/D	0.34	96.54	0.34	N/D	0.34	96.31	0.38	N/D	0.38
F23	97.92	0.36	N/D	0.38	96.97	0.30	N/D	0.30	96.56	0.29	N/D	0.29	96.34	0.30	N/D	0.30
F5	97.45	0.29	N/D	0.29	97.29	0.30	N/D	0.30	97.03	0.31	N/D	0.31	97.10	0.36	N/D	0.36

Table 32. Change in chemical stability after 3 freeze/thaw cycles, and after 3, 7, or 14 days of storage at 45°C, as determined by CEX in formulations F1, F9, F10, F5, and F15-F23. %AP - Acidic peak content [%]; %MAIN - main peak content [%]; %BP - basic peak content [%].

	T0			Freeze/Thaw			Thermal Stress								
				After 3 cycles			3 days at 45°C			7 days at 45°C			14 days at 45°C		
	%AP	%MAIN	%BP	%AP	%MAIN	%BP	%AP	%MAIN	%BP	%AP	%MAIN	%BP	%AP	%MAIN	%BP
F1	13.8	71.9	14.3	13.6	72.0	14.4	12.1	73.4	14.5	13.8	71.3	14.9	24.8	59.8	15.3
F15	13.8	72.1	14.1	13.6	72.1	14.3	12.2	73.3	14.5	13.8	71.5	14.7	24.9	59.9	15.2
F16	13.8	72.2	14.0	13.7	72.3	14.0	12.2	73.5	14.3	14.0	71.6	14.4	26.0	59.0	15.0
F17	13.8	72.0	14.2	13.7	72.1	14.1	12.1	73.6	14.4	13.7	71.5	14.8	24.8	59.8	15.4
F9	13.8	72.0	14.2	13.6	72.0	14.4	12.0	73.7	14.3	13.6	71.8	14.6	24.7	60.2	15.1
F18	13.9	71.8	14.3	13.7	72.0	14.3	12.2	73.5	14.3	13.9	71.7	14.3	25.0	60.1	14.8
F19	13.8	71.9	14.2	13.8	72.1	14.1	12.3	73.5	14.2	14.2	71.5	14.3	25.5	60.0	14.5
F20	13.8	72.0	14.2	13.8	72.2	14.1	12.0	73.6	14.3	13.6	71.9	14.5	24.8	60.2	15.0
F10	13.9	72.0	14.2	13.9	72.1	14.0	12.8	73.1	14.1	14.9	71.0	14.1	26.7	59.3	14.0
F21	13.9	72.0	14.1	13.9	72.2	14.0	12.7	73.3	14.0	15.2	70.9	13.9	26.6	59.5	13.9
F22	13.8	72.1	14.1	13.9	72.3	13.9	13.1	73.0	13.9	15.7	70.8	13.5	29.2	57.5	13.3
F23	13.8	72.1	14.1	13.9	72.3	13.9	12.8	73.2	14.0	15.2	71.0	13.9	27.1	59.0	13.9
F5	13.8	70.0	16.3	13.6	70.0	16.4	15.6	68.2	16.2	17.0	66.8	16.2	28.6	55.3	16.0

Table 33. Change in purity (CE non-reduced; main peak area [%]) in formulationf F1, F9, F10, F5 and F15-F23.

Formulation	T0	Freeze/Thaw	Thermal Stress		
		After 3 cycles	3 days at 45°C	7 days at 45°C	14 days at 45°C
	Purity	Purity	Purity	Purity	Purity
F1	97.8	97.7	96.9	95.3	93.5
F15	97.8	97.7	97.0	95.4	93.4
F16	97.8	97.7	97.0	95.4	93.2
F17	97.8	97.7	96.8	95.3	93.3
F9	97.8	97.7	96.9	95.3	92.7
F18	97.7	97.6	96.9	95.0	92.2
F19	97.7	97.6	96.8	94.9	91.7
F20	97.7	97.9	96.8	94.9	92.1
F10	97.8	97.9	96.8	94.8	91.8
F21	97.8	97.9	96.8	94.8	91.5
F22	97.8	97.9	96.5	94.5	91.0
F23	97.7	97.9	96.6	94.7	91.1
F5	97.6	98.0	97.5	96.7	95.4

Formulations F16, F17, F19, F20, F22, and F23 showed a reduced percentage of dimer formation and total aggregates if compared to F1, F9, and F10, as well as if compared to the prior art formulation F5 (cf. data in Table 30 after 2 freeze/thaw cycles and after freeze/thaw 3 cycles). Moreover, formulations F15, F16, F18, and F19, a reduced percentage of dimer formation and total aggregates after 14 days at 45°C if compared to F1, and F9, respectively (cf. Table 31, data for storage at 40°C). Formulation 23 showed a reduced percentage of dimer formation and total aggregates as compared to both formulation F10 and prior art formulation F5. All tested formulations showed a superior chemical stability (reduced percentage acidic peak content, and higher main peak content as determined by CEX) under thermal stress if compared to prior art formulation F5 (cf. data for 7 days and 14 days at 45°C in Table 32).

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Claims

1. An aqueous pharmaceutical composition comprising 1-50 mg/ml of a human or humanized full-length IgG_4 in a formulation selected from

   (i) a formulation comprising 10-25 mM histidine buffer, 130-180 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5; or

   (ii) a formulation comprising 25-30 mM phosphate buffer, 5-10 % (w/v) of a polyol, and 0.001-0.5 % (w/v) of a surfactant, pH 5.0-6.9; or

   (iii) a formulation comprising 10-25 mM histidine buffer, 10-50 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5.

2. The pharmaceutical composition of claim 1, wherein said pharmaceutical composition comprises 5-50 mg/ml, preferably 10-45 mg/ml, more preferably 15-40 mg/ml, even more preferably 20-30 mg/ml of said human or humanized full-length IgG_4.

3. The pharmaceutical composition of claim 1 or 2, wherein the IgG_4 is a humanized IgG_4.

4. The pharmaceutical composition of any one of claims 1-3, wherein the human or humanized full-length IgG_4 is natalizumab.

5. The pharmaceutical composition of any one of claims 1-4, wherein said formulation comprises 0.005-0.1 % (w/v), preferably 0.01-0.05 % (w/v), and more preferably 0.02 % (w/v) surfactant.

6. The pharmaceutical composition of any one of claims 1-5, wherein the surfactant is a nonionic surfactant.

7. The pharmaceutical composition of claim 6, wherein the nonionic surfactant is selected from polysorbate (Tween), poloxamer (Pluronic), polyethylene glycol alkyl ether (Brij), polyethylene glycol octylphenyl ether (Triton X-100), polypropylene glycol alkyl ether, glucoside alkyl ether, glycerol alkyl ester, and dodecyldimethylamine oxide;

   preferably wherein the nonionic surfactant is polysorbate or poloxamer;

more preferably wherein the nonionic surfactant is polysorbate 80, polysorbate 20, or poloxamer 188.

8. The pharmaceutical composition of claim 6, wherein the nonionic surfactant is polysorbate, preferably, wherein the nonionic surfactant is polysorbate 80.

9. The pharmaceutical composition of any one of claims 1-8, wherein formulation (i) comprises 10-20 mM histidine buffer; preferably 10-15 mM histidine buffer, more preferably 10-12 mM histidine buffer, and most preferably 10 mM histidine buffer.

10. The pharmaceutical composition of any one of claims 1-9, wherein said histidine in formulation (i) is L-histidine.

11. The pharmaceutical composition of any one of claims 1-10, wherein formulation (i) comprises 140-170 mM sodium chloride, preferably 145-165 mM sodium chloride, more preferably 150-160 mM sodium chloride, and most preferably 150 mM sodium chloride.

12. The pharmaceutical composition of any one of claims 1-11, wherein in formulation (i) the pH is 5.1-6.4, preferably pH is 5.1-6.3, more preferably the pH is 5.1-6.2, more preferably the pH is 5.1-6.1, more preferably pH is 5.2-6.0, more preferably pH is 5.3-5.9, more preferably pH is 5.4-5.8, even more preferably pH is 5.5-5.7, and most preferably formulation (i) has a pH of 5.7.

13. The pharmaceutical composition of any one of claims 1-12, wherein formulation (i) further comprises 5-30 mM methionine, preferably 6-25 mM methionine, more preferably 7-20 mM methionine, more preferably 8-15 mM methionine, more preferably 9-12 mM methionine, and most preferably 10 mM methionine.

14. The pharmaceutical composition of claim 13, wherein the methionine is L-methionine.

15. The pharmaceutical composition of any one of claims 13 or 14, wherein formulation (i) further comprises 5-50 mM glycine, preferably 10-45 mM glycine, more preferably 15-40 mM glycine, more preferably 20-35 mM glycine, more preferably 25-30 mM glycine, and most preferably 30 mM glycine.

16. The pharmaceutical composition of any one of claims 1-15, wherein formulation (i) further comprises 1-5 mM of a phosphate buffer, preferably 2-4 mM of a

phosphate buffer, more preferably 2.5-3 mM of a phosphate buffer, and most preferably 2.5 mM of a phosphate buffer.

17. The pharmaceutical composition of claim 16, wherein said phosphate buffer is sodium phosphate buffer.

18. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (i) consists of 10 mM L-histidine buffer, 150 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.

19. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (i) consists of 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 150 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.

20. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (i) consists of 25 mM L-histidine buffer, 150 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.

21. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (i) consists of 10 mM L-histidine buffer, 150 mM NaCl, 10mM L-methionine, and 0.02 % (w/v) polysorbate 80, pH 5.7.

22. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (i) consists of 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 150 mM NaCl, 10mM L-methionine, and 0.02 % (w/v) polysorbate 80, pH 5.7.

23. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (i) consists of 25 mM L-histidine buffer, 150 mM NaCl, 10mM L-methionine, and 0.02 % (w/v) polysorbate 80, pH 5.7.

24. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (i) consists of 10 mM L-histidine buffer, 150 mM NaCl, 10mM L-methionine, 30 mM glycine, and 0.02 % (w/v) polysorbate 80, pH 6.1.

25. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (i) consists of 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 150 mM NaCl, 10mM L-methionine, 30 mM glycine, and 0.02 % (w/v) polysorbate 80, pH 6.1.

26. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (i) consists of 25 mM L-histidine buffer, 150 mM NaCl, 10mM L-methionine, 30 mM glycine, and 0.02 % (w/v) polysorbate 80, pH 6.1.

27. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (i) consists of 10 mM L-histidine buffer, 150 mM NaCl, and 0.2 % (w/v) polysorbate 80, pH 5.7.

28. The pharmaceutical composition of any one of claims 1-8, wherein in formulation (ii) said phosphate buffer is sodium phosphate buffer.

29. The pharmaceutical composition of any one of claims 1-8 or 28, wherein the polyol in formulation (ii) is selected from sorbitol, sucrose, mannitol, xylitol, and inositol; preferably wherein the polyol is sorbitol or sucrose, more preferably wherein the polyol is sorbitol.

30. The pharmaceutical composition of any one of claims 1-8 or 28-29, wherein formulation (ii) comprises 5 % (w/v) of said polyol.

31. The pharmaceutical composition of any one of claims 1-8 or 28-30, wherein in formulation (ii) pH is 5.5-6.8, preferably 5.7-6.7, more preferably 5.8-6.7, more preferably 5.8-6.5, more preferably 6.0-6.5, and most preferably a pH of 6.1-6.3.

32. The pharmaceutical composition of any one of claims 1-8 or 28-31, wherein formulation (ii) further comprises 5-30 mM arginine-HCl, preferably 6-25 mM arginine-HCl, more preferably 7-20 mM arginine-HCl, more preferably 8-15 mM arginine-HCl, more preferably 9-12 mM arginine-HCl, and most preferably 10 mM arginine-HCl.

33. The pharmaceutical composition of claim 32, wherein the arginine-HCl is L-arginine-HCl.

34. The pharmaceutical composition of any one of claims 32 or 33, wherein formulation (ii) further comprises 0.5-10 mM methionine, preferably 0.75-5 mM methionine, more preferably 1-3 mM methionine, more preferably 1.5-2.5 mM methionine, , and most preferably 2 mM methionine.

35. The pharmaceutical composition of claim 34, wherein the methionine is L-methionine.

36. The pharmaceutical composition of any one of claims 1-8 or 28-31, wherein formulation (ii) further comprises 10-100 mM NaCl, preferably 20-80 mM NaCl, more preferably 25-75 mM NaCl, more preferably 30-70 mM NaCl, more preferably 40-60 mM NaCl, more preferably 45-55 mM NaCl, and most preferably 50 mM NaCl.

37. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (ii) consists of 25-30 mM sodium phosphate buffer, 5 % (w/v) sorbitol, and 0.02 % (w/v) polysorbate 80, pH 6.5.

38. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (ii) consists of 25-30 mM sodium phosphate buffer, 5 % (w/v) sorbitol, 50 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7-6.5.

39. The pharmaceutical composition of claim 38, wherein the pH is 5.7.

40. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (ii) consists of 25-30 mM sodium phosphate buffer, 5 % (w/v) sorbitol, 10 mM L-arginine-HCl, and 0.02 % (w/v) polysorbate 80, pH 6.1.

41. The pharmaceutical composition of claim 40, further comprising 2 mM L-methionine.

42. The pharmaceutical composition of any one of claims 37-40, wherein formulation (ii) comprises 30 mM sodium phosphate.

43. The pharmaceutical composition of any one of claims 1-8, wherein formulation (iii) comprises 10-20 mM histidine buffer; preferably 10-15 mM histidine buffer, more preferably 10-12 mM histidine buffer, and most preferably 10 mM histidine buffer.

44. The pharmaceutical composition of any one of claims 1-8 or 43, wherein said histidine in formulation (iii) is L-histidine.

45. The pharmaceutical composition of any one of claims 1-8 or 43-44, wherein formulation (iii) comprises 15-40 mM sodium chloride, preferably 20-30 mM sodium chloride, more preferably 20-25 mM sodium chloride, and most preferably 20 mM sodium chloride.

46. The pharmaceutical composition of any one of claims 1-8 or 43-45, wherein in formulation (iii) the pH is 5.1-6.4, preferably pH is 5.1-6.3, more preferably the

pH is 5.1-6.2, more preferably the pH is 5.1-6.1, more preferably pH is 5.2-6.0, more preferably pH is 5.3-5.9, more preferably pH is 5.4-5.8, even more preferably pH is 5.5-5.7, and most preferably formulation (iii) has a pH of 5.7.

47. The pharmaceutical composition of any one of claims 1-8 or 43-46, wherein formulation (iii) further comprises 1-5 mM of a phosphate buffer, preferably 2-4 mM of a phosphate buffer, more preferably 2.5-3 mM of a phosphate buffer, and most preferably 2.5 mM of a phosphate buffer.

48. The pharmaceutical composition of claim 47, wherein said phosphate buffer is sodium phosphate buffer.

49. The pharmaceutical composition of any one of claims 1-8 or 43-48, wherein formulation (iii) further comprises 5-30 mM methionine, preferably 6-25 mM methionine, more preferably 7-20 mM methionine, more preferably 8-15 mM methionine, more preferably 9-12 mM methionine, and most preferably 10 mM methionine.

50. The pharmaceutical composition of claim 49, wherein the methionine is L-methionine.

51. The pharmaceutical composition of any one of claims 49 or 50, wherein formulation (iii) further comprises 5-50 mM glycine, preferably 10-45 mM glycine, more preferably 15-40 mM glycine, more preferably 20-35 mM glycine, more preferably 25-30 mM glycine, and most preferably 30 mM glycine.

52. The pharmaceutical composition of any one of claims 1-8 or 43-46, wherein formulation (iii) further comprises 5-10 % (w/v) of a polyol, preferably 5-9 % (w/v) of a polyol, more preferably 5-8 % (w/v) of a polyol, more preferably 5-7 % (w/v) of a polyol, more preferably 5-6 % (w/v) of a polyol, and most preferably 5 % (w/v) of a polyol.

53. The pharmaceutical composition of claim 52, wherein the polyol is selected from sorbitol, sucrose, mannitol, xylitol, and inositol; preferably wherein the polyol is sorbitol or sucrose, more preferably wherein the polyol is sorbitol.

54. The pharmaceutical composition of claim 53, wherein formulation (iii) comprises 5 % (w/v) of sorbitol.

55. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (iii) consists of 10 mM L-histidine buffer, 20 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.

56. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (iii) consists of 10 mM L-histidine buffer, 20 mM NaCl, 5 % (w/v) of sorbitol, and 0.02 % (w/v) polysorbate 80, pH 5.7.

57. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (iii) consists of 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 20 mM NaCl, and 0.02 % (w/v) polysorbate 80, pH 5.7.

58. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (iii) consists of 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 20 mM NaCl, 10mM L-methionine, and 0.02 % (w/v) polysorbate 80, pH 5.7.

59. The pharmaceutical composition of any one of claims 1-4, wherein said formulation (iii) consists of 10 mM L-histidine buffer, 2.5 mM sodium phosphate, 20 mM NaCl, 10mM L-methionine, 30 mM glycine, and 0.02 % (w/v) polysorbate 80, pH 6.1.

60. The pharmaceutical composition of any one of claims 1-59, wherein the conductivity of said pharmaceutical composition is at least 2.5 mS/cm, preferably at least 2.6 mS/cm.

61. An article of manufacture, comprising a pharmaceutical composition according to any one of claims 1-60 and instructions for use of said composition, preferably wherein said pharmaceutical composition is contained in a pre-filled vial or in a pre-filled syringe, more preferably wherein said pharmaceutical composition is contained in a pre-filled vial.

62. A method for stabilizing a human or humanized full length IgG_4, comprising combining 1-50 mg/ml of a human or humanized full-length IgG_4 with a formulation selected from

(i) a formulation comprising 10-25 mM histidine buffer, 130-180 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5; or

(ii) a formulation comprising 25-30 mM phosphate buffer, 5-10 % (w/v) of a polyol, and 0.001-0.5 % (w/v) of a surfactant, pH 5.0-6.9; or

(iii) a formulation comprising 10-25 mM histidine buffer, 10-50 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5;

thereby preparing a stable aqueous pharmaceutical composition.

63. A method for producing an aqueous pharmaceutical composition according to any one of claims 1-60, comprising combining 1-50 mg/ml of a human or humanized full length IgG_4 with a formulation selected from

(i) a formulation comprising 10-25 mM histidine buffer, 130-180 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5; or

(ii) a formulation comprising 25-30 mM phosphate buffer, 5-10 % (w/v) of a polyol, and 0.001-0.5 % (w/v) of a surfactant, pH 5.0-6.9; or

(iii) a formulation comprising 10-25 mM histidine buffer, 10-50 mM sodium chloride, and 0.001-0.5 % (w/v) of a surfactant, pH 5.1-6.5.

64. The method of claim 62 or claim 63, wherein the human or humanized full-length IgG_4 is added in an amount of 5-50 mg/ml, preferably 10-45 mg/ml, more preferably 15-40 mg/ml, even more preferably 20-30 mg/ml of said human or humanized full-length IgG_4.

65. The method of any one of claims 62-64, wherein the IgG_4 is a humanized IgG_4.

66. The method of any one of claims 62-65, wherein the human or humanized full-length IgG_4 is natalizumab.

67. The method of any one of claims 62-66, wherein the formulation added to the human or humanized full-length IgG_4 is as further defined in claims 5-59.

68. The method of any one of claims 62-67, wherein the final stable aqueous pharmaceutical composition has a conductivity of at least 2.5 mS/cm, preferably at least 2.6 mS/cm.

69. An aqueous pharmaceutical composition according to any one of claims 1-60 for use in the treatment of multiple sclerosis or Crohn's disease.