IMMUNOGLOBULIN COMPOSITION AND METHOD OF GENERATING AN AEROSOL

Field of the invention

The present invention relates to a liquid aqueous composition comprising an immunoglobulin (Ig) and serine, a method of generating an aerosol, and an aerosol comprising particles comprising or essentially consisting of a composition as disclosed herein. The present invention further relates to the use of serine for increasing one or more properties selected from palatability, protein melting temperature, fine particle fraction, total output rate and delivered dose of a composition or aerosol as disclosed herein compared to a comparative composition or aerosol that does not comprise serine.

Background of the invention

Immunoglobulins (Ig) are components of human blood plasma which play an important role in immunological reactions. These specific immune proteins are synthesized by B-lymphocytes and found in blood plasma, lymph and other body secretions of all vertebrates. Immunoglobulins constitute approximately 20% of the plasma proteins in humans. One of the major challenges in the formulation of an immunoglobulin preparation lies in the fact that immunoglobulin dissolved in aqueous solution tend to aggregate and form precipitates if not sufficiently stabilised with appropriate additives. Carbohydrates are sometimes used as stabilizers. However, increasing concentrations of carbohydrates are associated with poor tolerability, in particular in the treatment of patients with impaired kidney function (e.g. diabetes patients).

With regard to the stabilisation of monomeric immunoglobulin, particularly good results have been achieved by using a basic or non-polar amino acid as a stabiliser. As for example disclosed in WO 2005/049078, the addition of basic or non-polar amino acids and the adjustment of the pH of the final preparation have been found to markedly decrease the formation of aggregates and thus increase the stability of those preparations, particularly at ambient temperature. Notably, the aforementioned prior art does not contain any information on the amino acid serine, which also belongs to another group, namely the group of polar, uncharged amino acids.

Generally, immunoglobulin preparations are administered intravenously or subcutaneously. Several commercial formulations are available for these administration routes, such as the applicant's immunoglobulin G (IgG) formulation Privigen® that is used to treat primary immunodeficiency disorder (PID) in humans with that condition and is normally administered intravenously. Subjects with PID have an immune system that does not function correctly and is characterized by an antibody deficiency. This means that subjects with PID are more likely to get and become very sick from infections. This also includes a higher risk of developing chronic lung diseases, for example. Depending on the country Privigen is also marketed for the treatment of chronic inflammatory demyelinating polyneuropathy (CIDP), secondary immunodeficiency disorder (SID), and idiopathic (autoimmune) thrombocytopenic purpura (ITP). A 20% Immune Globulin (SCIg) derived from plasma may be administered subcutaneously. Applicant's Hizentra® is marketed for treatment of PID, CIDP, and SID depending on the country.

Chronic lung diseases, in particular those that involve exacerbations where infections are the main driver, are characterized by difficulty for a subject to exhale the air in their lungs fully. Patients with such a chronic lung disease have shortness of breath due to difficulty exhaling all the air from the lungs. Because of damage to the lungs or narrowing of the airways inside the lungs, exhaled air comes out more slowly than normal. At the end of a full exhalation, an abnormally high amount of air may still linger in the lungs. Chronic obstructive pulmonary disease (COPD) and non-cystic fibrosis bronchiectasis (NCFB) are examples of such chronic lung diseases. COPD is characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients (Vestbo *et al.*, *American Journal of Respiratory and Critical Care Medicine* **2013**, 187(4), 347-365). NCFB is characterized by pathological dilation of the airways - clinically identified by radiographic demonstration of airway enlargement, i.e. by a CT scan (Flume *et al.*, *Lancet* **2018**, 392(10150), 880-890). Exacerbations are considered to be key events in the progression of NCFB (Chalmers *et al.*, *American Journal of Respiratory and Critical Care Medicine* **2018**, 197(11), 1410-1420).

Acute exacerbations of respiratory symptoms often occur in patients with chronic lung diseases, such as COPD and NCFB. These acute exacerbations may be triggered by infection with bacteria or viruses (which may coexist). During exacerbations, there is a flare-up of inflammation, increased hyperinflation and gas trapping, reduced expiratory flow, and increased dyspnea. Other medical conditions, such as pneumonia may aggravate an exacerbation of e.g. COPD. An acute exacerbation of COPD is defined by the global initiative for chronic obstructive lung disease

(GOLD) as an acute worsening of the patient's respiratory symptoms that is beyond normal day-to-day variations that results in additional therapy medication. The rate at which exacerbations occur varies greatly between patients. Chronicity of exacerbations in patients with COPD support tissue remodeling of the airways and contribute to the aggravation of the disease. These acute exacerbations correlate with a high degree of systemic inflammation and immune activation. As COPD severity worsens, the frequency of exacerbations increases. In turn, acute exacerbations likely increase the progression of COPD, and additionally, it is likely that the inflammatory state created by the acute exacerbations increases susceptibility to additional, recurrent acute exacerbations. This leads to a vicious cycle driving progression of COPD.

An exacerbation of NCFB may be defined as the acute worsening of one or more symptoms of NCFB beyond normal day-to-day variations, for example the requirement of antibiotics in the presence of one or more symptoms such as increasing cough, increasing sputum volume, or worsening sputum purulence. A severe exacerbation may be defined as requiring unscheduled hospitalization or an emergency department visit (Chalmers *et al.*, *American Journal of Respiratory and Critical Care Medicine* **2018**, 197(11), 1410-1420).

Patients with chronic lung diseases, such as COPD or NCFB, are likely to present with recurrent respiratory tract infections, which can trigger an acute exacerbation. The most common causes of acute exacerbations of COPD are viral infections of the upper respiratory tract and the tracheobronchial tree. The most common viruses detected during COPD exacerbations are human rhinoviruses (HRV) (Mohan A *et al.*, *Respirology* **2010**, 15(3), 536-542), which are associated with an outgrowth of the bacterial airway microbiome (Molyneaux *et al.*, *American Journal of Respiratory and Critical Care Medicine* **2013**, 188(10), 1224-1231). Bacterial flora in COPD is generally highly variable. Many different bacteria have been associated with COPD. However, the most pathogenic ones include Haemophilus influenza, Streptococcus pneumonia, Moraxella catarrhalis, Haemophilus parainfluenzae and Staphylococcus aureus. In addition, Pseudomonas aeruginosa (PA) has been described to be one of the most harmful bacteria found in patients with excessively severe airflow obstruction in stable COPD and during exacerbations (Hassett *et al.*, *Journal of Microbiology* **2014**, 52(3), 211-226).

Treatments for COPD are based on inhaled corticosteroids (ICS), inhaled bronchodilators including long-acting beta2-agonists, and anticholinergics including long-acting muscarinic receptor antagonists, and combinations of these. For example, severe COPD with a high risk of exacerbations is commonly treated with a combination of all three classes of drugs. These therapies reduce exacerbations but patients taking maximum inhaled therapy continue to experience exacerbations

and therefore new therapeutic approaches are needed. Indeed, ICS therapy is associated with side effects, including high risk of pneumonia, oral candidiasis, hoarse voice and skin bruising. Other side effects include increased risk of new-onset diabetes, diabetes progression, cataracts and tuberculosis. Long-term use is also associated with increased risk of bone fractures in COPD patients (Miravitlles *et al.*, *Respiratory Research* **2017**, 18, 198). In particular, ICS therapy only modestly reduces the frequency of exacerbations and clinical trials report an increased risk of pneumonia with ICS use in COPD. This may be because ICSs appear to reduce antiviral immunity, leading to mucus hypersecretion and increased lung bacterial loads (Singanayagam *et al.*, *Nature Communications* **2018**, 9(1), 2229).

In COPD patients with chronic bronchitis, a phosphodiesterase-4 enzyme inhibitor (e.g. roflumilast) can be added to the selected treatment. Roflumilast is a non-steroid, anti-inflammatory active substance designed to target both the systemic and pulmonary inflammation associated with COPD. It is indicated for maintenance treatment of severe COPD associated with chronic bronchitis in adult patients with a history of frequent exacerbations as an adjunct therapy to bronchodilator treatment.

Acute exacerbations of COPD are currently managed with pharmacological therapies including bronchodilators, ICS and antibiotics. ICS therapy is associated with side effects, as discussed above. Antibiotics are used to treat bacterial respiratory tract infections, in order to reduce occurrence and severity of exacerbations. Macrolides also have an anti-inflammatory effect and may be used in patients with severe COPD and a history of frequent exacerbations. However, long-term macrolide therapy is associated with risk of microbial resistance and cardiovascular adverse effects. There are currently no agents for treating viral infections, such as rhinovirus infections, in COPD.

There are no treatments available for NCFB. Acute exacerbations of NCFB are commonly treated with antibiotics, to eliminate underlying respiratory tract infection. Some NCFB patients receive prophylactic antibiotic therapy to prevent exacerbations. However, the efficacy of such therapy has not been proven.

It is generally acknowledged that the treatment or prevention of chronic lung diseases preferably involves topical administration of drugs in the target tissue. In this regard, topical administration of immunoglobulin preparations, more specifically administration to the respiratory tract has been suggested.

For example, US 4,994,269 describes a method for topical administration of antibodies to P. aeruginosa. The antibodies can be administered in the form of an aerosol, e.g. via application to the nose, as an aerosol to the lungs or via intratracheal administration.

WO 92/01473 describes a method which comprises administering into the lower respiratory tract of a susceptible host a small particle (< 2 µm) aerosol of a mixture of specific monoclonal antibodies directed against the various protective antigenic sites present on a major protective viral surface antigen(s).

WO 2011/098552 describes methods for the preparation of an aerosol of immunoglobulin single variable domains wherein the amount of aggregate formation is significantly reduced.

US 9'084'777 B describes stable antibody-containing formulations which have been obtained from high concentration antibody-containing formulation by means of lyophilization. The formulations can be free of reducing sugars, sugar alcohols or polysaccharides as excipients. Nevertheless, the major drawback of this approach is the requirement of reconstituting the solution.

EP 2 735 315 B describes protein-comprising formulations which are stable against photostress. Such solutions are obtained by adding additional stabilizing agents, such as argininamide, arginine ethyl ester, homoarginine or valinamide.

WO 2015/150510 A1 describes a method of nebulizing a composition containing immunoglobulin. The composition can comprise proline as stabilizer and agent for reducing the viscosity.

WO 2020/109621 A1 describes the prevention or treatment of acute exacerbations in a human subject with a chronic lung disease by localized administration of a composition comprising polyclonal immunoglobulin to the respiratory tract. The composition can comprise stabilizers such as proline.

Although these documents suggest several methods of applying different types of antibodies, nebulizing polyclonal immunoglobulins (Ig), in particular polyclonal immunoglobulin G (IgG), still poses a challenge for optimal nebulization and administration.

In particular, plasma derived immunoglobulin material has limited resources, and such immunoglobulins are highly valuable drug substances. Therefore, there is a need of further optimizing the nebulization in order to limit the amount of immunoglobulin necessary for administration.

In addition, there is a need to improve the nebulization performance of an immunoglobulin solution, for example, to reduce the duration of inhalation required to administer a required dose. Furthermore, there is a desire for improved IgG-containing aerosol properties, to achieve a more targeted application of the IgG to the desired lung areas during inhalation.

Hence, there is still a need in the art for stable compositions comprising polyclonal immunoglobulin (Ig) featuring improved nebulization parameters, i.e. improved suitability for nebulization, for IgG containing aerosols with improved suitability for inhalation, and for an improved method of nebulizing polyclonal immunoglobulin (Ig), e.g. IgG, IgA, IgM, or combinations thereof, in a particularly fast and efficient manner.

Therefore, it is an object of the present invention to provide a stable composition comprising polyclonal immunoglobulin (Ig), the composition having advantageous nebulization properties, i.e. improved suitability for nebulization. At the same time, the composition, in particular, when presented as a stable liquid formulation, should have at least acceptable immunoglobulin stability during long term storage.

It is a further object of the present invention to provide an improved method of generating an aerosol by nebulizing a composition comprising polyclonal immunoglobulin (Ig).

It is a further object of the present invention to provide an immunoglobulin-containing aerosol with improved suitability for inhalation. The aerosol generated by the method should have improved suitability for delivery of polyclonal immunoglobulin (Ig) to the respiratory tract of a patient.

Summary of the invention

The objects are solved by the subject matter according to the independent claims. Preferred embodiments are defined in the dependent claims.

The present disclosure is based on the inventors' identification of a composition comprising polyclonal immunoglobulin (Ig) and serine, in particular L-serine, as a stabilizer. According to a first aspect, the present invention thus relates to a liquid aqueous composition, in particular suitable for inhalation, even more preferably suitable for inhalation when nebulized, comprising a polyclonal immunoglobulin (Ig) and a stabilizer, wherein the stabilizer comprises serine, in particular L-serine.

The inventors of the present invention investigated what were known to be key stabilizing attributes for the immunoglobulin (Ig), including pH, immunoglobulin (Ig) concentration, viscosity, molecular integrity of the immunoglobulin (Ig) protein and the excipient's ability to stabilize the immunoglobulin (Ig) protein. Inventors also evaluated the aerosol characteristics of the formulations in combination with a number of delivery devices and conducted stability programs for key candidates. At it, the inventors of the present invention have surprisingly found that by adding serine, and in particular L-serine, a relatively low viscosity of the polyclonal immunoglobulin (Ig) composition can be achieved even if the concentration of immunoglobulin (Ig) is high. Although serine has been reported to have a stabilizing effect on protein preparations, it is surprising that it is equally stabilizing as proline with similar viscosity reducing ability, its effect of reducing the viscosity of a polyclonal immunoglobulin preparation as a sole agent has however nowhere been considered so far. It has also surprisingly been found that the use of serine, in particular L-serine, in immunoglobulin (Ig) compositions results in a number of further advantages, which are described in more detail below and are particularly advantageous in the nebulization of such compositions to generate an aerosol. In the context of nebulizing polyclonal immunoglobulins (Ig), the first experiments were aimed at modifying existing nebulization devices such that they would be able to deliver proline stabilized IgG formulation as it was not apparent that changing the excipient would be beneficial in terms of delivery. Importantly, serine was found to be the best candidate taking all nebulization related performances into account as will be described below.

Specifically, the inventors of the present invention surprisingly found that the presence of serine has the combinatory beneficial effect (i) of stabilizing immunoglobulin (Ig) and thus allowing to obtain a composition having a very high stability over a relatively long period of time, such as at least one year, in particular at least two years, (ii) of providing a low viscosity, thus allowing administration of the preparation in a fast and simple manner, and (iii) of improving the nebulization properties of the immunoglobulin (Ig) composition.

In addition, it was found that serine is safe upon inhalation which has not been demonstrated before. The present inventors demonstrated that nebulized L-serine has a favourable safety profile alone and in combination with IgG. Therefore, L-serine is suitable as a stabilizer in IgG compositions for administration by inhalation.

The term "viscosity" as used in the context of the present invention means dynamic viscosity. The SI physical unit of dynamic viscosity is millipascal second (mPa·s). The viscosity can, for example, be determined by a falling sphere viscosimeter ("Kugelfallviscosimeter") according to Höppler in

accordance with the European Pharmacopoeia Version 6.0 at 2.2.49 and the requirements of DIN 53015. Thereby, the rolling time of a ball or sphere is a tube or capillary of defined dimensions and having a defined slope is determined. Based on the rolling time, the viscosity of the liquid in the tube or capillary can be determined. The values given in the present disclosure have been determined by the above using a microviscosimeter of the type AMV200 (of Anton Paar GmbH, Graz, Austria). The measurements have been made at a temperature of 20.0°C +/- 0.1°C.

Thus, according to a preferred embodiment, the composition of present invention is a stable liquid formulation which is directly suitable for nebulization. Long term stability and safety of such a formulation has been demonstrated by the inventors. The formulation is therefore suitable for pharmaceutical use, i.e. as a medicament, in particular as a ready-to-use formulation for nebulization and/or inhalation.

According to a preferred embodiment, the immunoglobulin (Ig) is selected from at least one of polyclonal immunoglobulin G (IgG), polyclonal immunoglobulin A (IgA), and polyclonal immunoglobulin M (IgM). The aforementioned immunoglobulin classes, i.e. IgG, IgA and IgM, are of particular importance: Human immunoglobulin G (IgG) represents the most abundant immunoglobulin in plasma and is responsible for e.g., toxin neutralization, complement activation and opsonisation. Immunoglobulin A (IgA) represents the main antibody class in external secretions such as saliva, tears and mucus of the respiratory and intestinal tracts. IgA forms one of the first lines of defense against bacterial and viral pathogens. Immunoglobulin M (IgM) is by far the physically largest antibody in the human circulatory system, appears early in the course of an infection and usually reappears, to a lesser extent, after further exposure.

According to a particular embodiment, the immunoglobulin (Ig) is at least 95% polyclonal IgG, preferably at least 98% polyclonal IgG.

Thus, the present invention is particularly advantageous for providing improved IgG-containing aerosols, to achieve a more targeted application of the IgG to the desired lung areas during inhalation.

According to a further preferred embodiment, the immunoglobulin (Ig) is human plasma-derived polyclonal immunoglobulin G (hIgG). IgG purified from human plasma is used for prophylactic prevention of infections in immunodeficient patients, replacement therapy for antibody deficiencies in patients, and the treatment of conditions relating to immune deficiencies, inflammatory and autoimmune diseases and acute infections in patients. Polyclonal human plasma-derived

immunoglobulin G (hIgG) is particularly suitable for use in the prevention or treatment of an acute exacerbation in a subject with a chronic lung disease, typically COPD and/or NCFB, by treating and/or preventing one or more respiratory tract infections.

The polyclonal immunoglobulin (Ig) of present invention according to preferred embodiments is either normal human immunoglobulin or a hyperimmune immunoglobulin. Most preferred is the normal human immunoglobulin.

Preferably, the plasma from multiple donors is pooled in order to maximize the diversity of target antigen specificities, for example from more than 100 donors, preferably from more than 500 donors, even more preferably from more than 1,000 donors.

Typically, the plasma pools are subjected to ethanol fractionation, followed by several purification steps, such as further precipitation steps and/or column chromatography steps, as well as steps to inactivate and remove viral and other pathogens such as nanofiltration or solvent/detergent treatment.

Alternatively, the polyclonal immunoglobulin can be produced recombinantly, e.g. from libraries comprising the human immune repertoire.

As already stated above, an aspect of the invention is the use of a single agent for increasing at least one of the immunoglobin long-term stability, proportion of fine particle fraction (FPF) and fine particle dose (FPD), total output rate (TOR), delivered dose (DD), delivered dose percent (DD%) and/or palatability of a liquid aqueous immunoglobulin composition, wherein the single agent is serine, preferably L-serine. As sufficient stabilization is achieved by the presence of serine, and in particular L-serine, the addition of other stabilizers can be avoided. The inventors surprisingly found that serine cannot only stabilize IgG in solution, at high concentrations of IgG serine can be both stabilizing and viscosity reducing on its own, i.e. without combination of other stabilizers, like amino acids or sugars. Thus, according to a preferred embodiment, the stabilizer essentially consists of serine. Put differently, the composition is preferably free of stabilizers for the protein, except for serine. This also allows the complexity of the composition to be reduced and the beneficial properties of serine, in particular of L-serine, to be particularly pronounced.

When using serine as a stabilizing agent, it is particularly preferred to use L-serine. L-Serine is a non-essential amino acid and normal body constituent with a plasma concentration of about 0.1 mM, which can be easily utilized by any body cell for protein synthesis, energy conversion and

biosynthesis of several other amino acids. No adverse effects due to L-serine have been described based on data available for the oral and intravenous route. L-serine is normally present in the human body and has a very favorable toxicity profile. The present inventors demonstrated that nebulized L-serine has a favourable safety profile alone and in combination with IgG, when inhaled for example by cynomolgus monkeys. Therefore, also from a safety perspective, L-serine is suitable as a stabilizer in IgG compositions for administration by inhalation.

According to a preferred embodiment, the composition is essentially free of amino acids other than serine. Serine was found to be safe upon inhalation delivery to non-human primates.

Carbohydrates are sometimes used as stabilizers. However, increasing concentrations of carbohydrates are associated with poor tolerability, in particular in the treatment of patients with impaired kidney function such as diabetes patients. Thus, according to a preferred embodiment, the composition is essentially free of at least one of carbohydrates and polyols. The absence of carbohydrates has a beneficial effect on tolerability of the composition.

According to a preferred embodiment, the composition of present invention is preferably not lyophilized and not a reconstituted solution obtained by adding water suitable for injection to an antibody-containing lyophilized pharmaceutical formulation. The composition of present invention is preferably a stable liquid formulation which is directly suitable for nebulization.

According to a preferred embodiment, the composition of present invention does neither include argininamide nor valinamide.

According to a preferred embodiment, the composition of present invention does not include any surfactant like polysorbate 20, polysorbate 40, polysorbate 60 or polysorbate 80. If present, the concentration of a surfactant should be low, preferably not exceed 100 µg/mL, 30 µg/mL or 20 µg/mL. In general, the concentration of a surfactant should be low, in order to avoid undesirable formulation behavior such as foaming, in particular in the method of generating an aerosol. Typically, the composition of present invention comprises only trace amounts of surfactant, for example as process-based impurity.

The invention relates to administration of the composition of the invention to the respiratory tract of the subject. For such administration to the respiratory tract, it is preferred to use relatively high concentrations of immunoglobulin (Ig). Relatively high concentrations are useful to minimize the volume to be administered as much as possible, for example when administered by nebulizer to

keep the nebulization time as short as possible. Keeping nebulization time as short as possible is particularly useful for maintaining subject compliance. On the other hand, high concentrations of immunoglobulins are associated with high viscosity and thus with challenges to nebulize such viscous solutions. Thus, according to a preferred embodiment, the concentration of the immunoglobulin (Ig) in the composition is equal to or lower than about 120 mg/mL, preferably in the range of 50 mg/mL to 120 mg/mL, more preferably 60 mg/mL to 110 mg/mL, even more preferably 60 mg/mL to 100 mg/mL, further preferably 60 mg/mL to 90 mg/mL, even further preferably 60 mg/mL to 80 mg/mL and most preferably about 70 mg/mL. Such relatively high concentrations of immunoglobulin (Ig) enable low fill volumes and short nebulization times and, thus, ensure therapeutic efficiency of the composition when used in a therapeutic method. On the other hand, the concentrations are low enough to allow for nebulization of viscous solutions.

Depending on the clinical dosing requirements, it can be beneficial to provide a less concentrated formulation. Therefore, according to another preferred embodiment the composition of the present invention has a concentration of the immunoglobulin (Ig) being equal to or lower than about 120 mg/mL, preferably in the range of 10 mg/mL to 120 mg/mL, more preferably 10 mg/mL to 110 mg/ mL, even more preferably 10 mg/mL to 80 mg/ mL, further preferably 10 mg/mL to 70 mg/mL, even further preferably 10 mg/ mL to 60 mg/mL, even further preferably 10 mg/ mL to 50 mg/mL, even further preferably 10 mg/ mL to 40 mg/mL, and even further preferably 10 mg/ mL to 30 mg/mL. Such composition may for example have a concentration of the immunoglobulin (Ig) of about 10 mg/mL, about 15 mg/mL, about 20 mg/mL, about 25 mg/mL, about 30 mg/mL, about 35 mg/mL, about 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL or about 60 mg/mL.

According to a preferred embodiment, the amount of serine, in particular of L-serine, added is such that the concentration of serine, in particular of L-serine, in the composition ranges from 100 to 400 mM, preferably 250 to 300 mM, and most preferably about 270 mM. Serine, and in particular L-serine, used in this concentration range sufficiently stabilizes the composition. In addition, the nebulization is more efficient than IgG formulations with proline.

According to a preferred embodiment, the composition has a pH in the range of from 4.0 to 6.0, preferably 4.5 to 5.0, most preferably a pH of about 4.8, which further contributes to the high stability of the liquid aqueous composition. In addition, the pH needs to be conducive to the lung and to nebulization.

The effect of serine, in particular of L-serine, to reduce the viscosity of the immunoglobulin (Ig) composition is of particular relevance for immunoglobulin (Ig) compositions containing high immunoglobulin (Ig) concentrations, specifically immunoglobulin (Ig) compositions having a mass-volume percentage equal to or lower than about 120 mg/mL, preferably in the range of 50 mg/mL to 120 mg/mL, more preferably 60 mg/mL to 110 mg/mL, more preferably 60 mg/mL to 100 mg/mL, even more preferably 60 mg/mL to 90 mg/mL, further more preferably 60 mg/mL to 80 mg/mL and most preferably about 70 mg/mL. As mentioned above, depending on the clinical dosing requirements, it can be beneficial to provide a less concentrated formulation and therefore the composition may have a concentration of the immunoglobulin (Ig) being equal to or lower than about 120 mg/mL, preferably in the range of 10 mg/mL to 120 mg/mL, more preferably 10 mg/mL to 110 mg/ mL, even more preferably 10 mg/mL to 80 mg/ mL, further preferably 10 mg/mL to 70 mg/mL, even further preferably 10 mg/ mL to 60 mg/mL, even further preferably 10 mg/ mL to 50 mg/mL, even further preferably 10 mg/ mL to 40 mg/mL, and even further preferably 10 mg/ mL to 30 mg/mL. Some or even all of the beneficial effects of serine disclosed herein may also be applicable in those slightly lower concentrated immunoglobulin (Ig) compositions.

According to a preferred embodiment, the composition has a viscosity in the range of from 2.3 to 3.0 mPa·s, determined by a falling sphere viscosimeter according to Höppler in accordance with the European Pharmacopoeia Version 6.0 at 2.2.49 and the requirements of DIN 53015 at a temperature of 20.0°C ± 0.1°C.

Having learned from the teaching of the present invention, a skilled person readily realizes how to choose the respective amounts of serine, in particular of L-serine, in order to achieve the viscosity aimed for. The use of serine, in particular L-serine, as stabilizer makes it possible to provide liquid aqueous immunoglobulin (Ig) compositions with such relatively low viscosities, even for relatively highly concentrated immunoglobulin (Ig) compositions, so that the compositions can be administered in a fast and simple manner. In particular, conventional means currently used for nebulization of immunoglobulin (Ig) preparations can be used to generate aerosols of the liquid aqueous immunoglobulin (Ig) compositions disclosed herein.

Surfactants can be added to the composition of the invention. These can help to control the rate of aggregation of polyclonal immunoglobulin in the composition, i.e. during storage and in the reservoir, and during nebulization, i.e. during and after passing the mesh of the nebulizer, hereby having an influence on the activity of the polyclonal immunoglobulin in the aerosol. Therefore, according to a preferred embodiment, the liquid aqueous compositions disclosed herein further

comprise at least one surfactant in an amount such that the composition has a surface tension of about 60 to 75 mN/m, preferably about 64 to 71 mN/m.

According to a further preferred embodiment, the at least one surfactant is polysorbate 80, preferrable at a concentration between 5 and 100 µg/mL, more preferred at a concentration between 8 and 30 µg/mL. Most preferred is a concentration of about 20 µg/mL.

A composition of the invention may include components in addition to the polyclonal immunoglobulin (Ig) and the serine, e.g. it may include one or more further pharmaceutical carrier(s) and/or excipient(s). Thus, according to one embodiment, the composition comprises at least one further pharmaceutically acceptable excipient. Pharmaceutically acceptable excipients serve to optimize the characteristics of the composition and/or the characteristics of the aerosol. Examples of such excipients are excipients for adjusting or buffering the pH, excipients for adjusting osmolality, antioxidants, surfactants, excipients for sustained release or prolonged local retention, taste-masking agents, sweeteners, and flavors. These excipients are used to obtain an optimal pH, osmolality, viscosity, surface tension and taste, which support the formulation stability, the aerosolization, the tolerability, and/or the efficacy of the formulation upon inhalation. A discussion of such components is available in reference Gennaro, Remington: The Science and Practice of Pharmacy, 20th edition (2000), ISBN: 0683306472.

According to a preferred embodiment, the liquid aqueous composition essentially consists of polyclonal immunoglobulin G (IgG) at a concentration of between 50 to 120 mg/mL and L-serine at a concentration of between 100 to 400 mM in water for infusion, wherein the pH of the aqueous composition is adjusted to 4.5 to 5.0.

According to an even preferred embodiment, the liquid aqueous composition essentially consists of polyclonal immunoglobulin G (IgG) at a concentration of about 70 mg/mL and L-serine at a concentration of about 270 mM in water for infusion, wherein the pH of the aqueous composition is adjusted to about 4.8.

The composition of the invention has an osmolality close to an isotonic formulation to avoid inducing any adverse reaction in the lung such as coughing reflexes. The composition has preferably an osmolality of between 150 to 550 mOsmol/kg, between 200 to 350 mOsmol/kg, between 210 to 300 mOsmol/kg, and most preferred between 280 to 310 mOsmol/kg.

Apart from a preferred viscosity, the use of serine, in particular of L-serine, provides the composition of the present invention with a high storage stability. The immunoglobulin (Ig) compositions disclosed herein are storage-stable over extended time periods. The term “storage-stability” as used herein refers to the maintenance of one or more features of the composition over the storage period. For example, storage-stability is indicated by the absence of immunoglobulin aggregation, which can, for example, be determined using size exclusion high-performance liquid chromatography (SEC-HPLC).

For example, size exclusion high-performance liquid chromatography (SEC-HPLC) analysis can be performed by injecting 200 µg/2 µL (IgG) or 100 µg/2 µL (IgA, IgM) into an Agilent Technologies 1260 Infinity™ HPLC system for size exclusion chromatography at a flow rate of 0.7 mL/min over a TSK gel G3000SWxl 7.8 mm ID x 30 cm column (Tosoh Bioscience). From the resulting chromatograms the relative contents of (i) immunoglobulin polymers and aggregates, (ii) monomers and dimers, as well as (iii) fragments, respectively, can be assessed.

According to one embodiment, the immunoglobulin (Ig) remains at least 80% monomeric during storage at 2°C to 8°C for at least one year, preferably for at least two years, as determined by SEC-HPLC.

According to one embodiment, the immunoglobulin (Ig) remains at least 96% monomeric/dimeric during storage at 2°C to 8°C or at 25°C for at least six months, as determined by SEC-HPLC.

According to a preferred embodiment, the composition is storage-stable in liquid form for at least 12 months when stored at a maximum temperature of 25°C. In a further preferred embodiment, the composition is storage-stable in liquid form for at least 24 months when stored at a maximum temperature of 25°C. In an even further preferred embodiment, the composition is storage-stable in liquid form for at least 30 months when stored at a maximum temperature of 25°C. The room temperature stability provides improved flexibility and convenience compared with other compositions that must be kept refrigerated.

According to a preferred embodiment, the immunoglobulin (Ig) monomer and dimer content of the composition remains above 95% during storage for at least 12 months when stored at a maximum temperature of 25°C. In a further embodiment, the immunoglobulin (Ig) monomer and dimer content of the composition remains above 95% during storage for at least 24 months when stored at a maximum temperature of 25°C. In one embodiment, the immunoglobulin (Ig) monomer and dimer content of the composition remains above 98% during storage for at least 12 months when

stored at a maximum temperature of 25°C. In a further embodiment, the immunoglobulin (Ig) monomer and dimer content of the composition remains above 98% during storage for at least 24 months when stored at a maximum temperature of 25°C.

According to a second aspect, the present disclosure relates to the prevention or treatment of a respiratory disease, by administration of a composition comprising polyclonal immunoglobulin (Ig) and serine as disclosed herein to the respiratory tract, in particular by direct application of an aerosolized composition comprising polyclonal immunoglobulin (Ig) and serine. Hence, according to a preferred embodiment, the composition is for use as a medicament, in particular for use in the treatment or prevention of a disease affecting the lungs of a subject, wherein the composition is administered as an aerosol, i.e. nebulized, to the respiratory tract of the subject. The nebulized composition is preferably inhaled via mouth.

The nebulization performance of the immunoglobulin solution is enhanced as described before and thus allows for, for example, a reduction of the duration of inhalation required to administer a required dose. Further, the IgG-containing aerosol properties are improved and thus allow for a more targeted application of the IgG to the desired lung areas during inhalation.

The invention also relates to a liquid aqueous composition, in particular as described herein, for use as a medicament. The liquid composition comprises a polyclonal immunoglobulin (Ig) and a stabilizer, wherein the stabilizer comprises serine, in particular L-serine.

The invention also relates to a liquid aqueous composition, in particular as described herein, for use in the treatment of respiratory diseases.

The invention also relates to a liquid aqueous composition, in particular as described herein, for use in the treatment of acute exacerbations in chronic lung diseases, such as chronic obstructive pulmonary disease (COPD) and non-cystic fibrosis bronchiectasis (NCFB).

The invention also relates to the use of a liquid aqueous composition, in particular as described herein, for the manufacture of a medicament for the treatment of respiratory diseases, in particular acute exacerbations in chronic lung diseases, such as chronic obstructive pulmonary disease (COPD) and non-cystic fibrosis bronchiectasis (NCFB).

According to additional preferred embodiments, the respiratory disease is selected from the group consisting of NCFB, COPD, Cystic Fibrosis (CF) and pneumonia. These patient populations lack

the ability to mount an effective antimicrobial, anti-viral and anti-fungal defences; have been infected or colonized with difficult to treat bacterial strains (e.g. antibiotic resistant strains) or the diseased tissue/disease state hinders the ability of the patient's immune system/exogenous therapy to effectively clear the pathogen.

The respiratory disease according to further preferred embodiments can be a primary and secondary immunodeficiency. These patients have an increased risk of catching respiratory tract infections due to their compromised immune systems.

Without wishing to be bound by theory, the proposed mechanism of action having polyclonal immunoglobulin (Ig), in particular polyclonal immunoglobulin G (IgG), delivered directly to the lung is a reduction in the pathogen-driven respiratory tract exacerbations by targeting pathogens such as viruses, bacteria or fungi in the respiratory tract, neutralizing viruses and bacterial toxins, blocking adherence of bacteria, i.e. preventing the bacteria forming biolayers or biofilms on the surface of the lung, and opsonizing pathogens and enhancing complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC). The latter are immunological effects to deplete those bacteria and viruses from the lung and the exacerbations that those bacteria cause.

The respiratory tract is divided into two main parts: the upper respiratory tract, consisting of the nose, nasal cavity, the pharynx and the portion of the larynx above the vocal folds (cords); and the lower respiratory tract, consisting of the portion of the larynx below the vocal folds, trachea, bronchi, bronchioles and the alveoli. The so-called tracheobronchial tree is a complex system that begins at the edge of the larynx, divides into two bronchi and continues into the lungs. The tracheobronchial tree is partitioned into 23 generations (divisions) of dichotomous branching, i.e. at each generation each airway is being divided into two smaller daughter airways. It extends from the trachea (generation 0) to the last order of terminal bronchioles (generation 23).

According to a further preferred embodiment, the composition is for use in the treatment or prevention of a disease affecting the lungs of a subject, wherein the composition is administered as an aerosol to the respiratory tract of the subject. The disease affecting the lungs may be selected from at least one of chronic obstructive pulmonary disease (COPD) and non-cystic fibrosis bronchiectasis (NCFB), Primary immunodeficiency (PID), Cystic Fibrosis (CF) or pneumonia. The to be treated patient population in particular lacks the ability to mount an effective antimicrobial/anti-viral/anti-fungal defence or has been infected/colonized with difficult to treat/antibiotic resistant strains or in which the diseased tissue/disease state hinders the ability of the patient's immune system/exogenous therapy to effectively clear the pathogen. This may also include patients with

secondary immunodeficiencies such as those with cancer who are very high risk for morbidity and mortality and for those who have received stem cell or solid organ transplants and are on chronic immunosuppressive therapy and at increased risk for infection.

In addition to the beneficial effect of directly targeting pathogens in the respiratory tract via inhalation, there can also be an increased patient convenience associated with the inhalation route. In contrast to intravenous administration or subcutaneous self-administration of immunoglobulin therapy, self-administration through inhalation of an aerosol immunoglobulin may be the preferred application, in particular at home. Consequently, direct administration to the respiratory tract may be more practical for the subject and therefore the subject may be more likely to comply with the treatment. Increased compliance reduces therapeutic failures, which can lead to acute exacerbations and hospitalization, for example.

Even if the immunoglobulin (Ig) concentration in the composition disclosed herein is relatively high, the present composition can be administered in a fast and simple manner due to its low viscosity and/or the favorable nebulization performance. In particular, conventional means suggested for nebulizing immunoglobulin (Ig) compositions of lower concentration or for solutions with other stabilizers than serine can be used to generate an aerosol of the compositions disclosed herein. In particular, the aerosol may be generated from a liquid aqueous composition using a nebulizer. Suitable liquid aqueous compositions are set out above.

Hence, according to a third aspect, the present invention relates to a method of generating an aerosol. The method comprises the steps of a) providing a composition as disclosed herein, and b) nebulizing the composition provided in step a) by means of a nebulizer, in particular a vibrating mesh nebulizer, thereby generating the aerosol.

Vibrating mesh nebulizers have been described in detail for example by J.N. Pritchard, *Therapeutic Delivery* **2018**, 9, 121-136.

Applying the method of the invention results in an aerosol in which the activity of the polyclonal immunoglobulin (Ig), in particular polyclonal IgG, is preferably at least 80% of the activity of the immunoglobulin in the composition filled into the nebulizer reservoir. Thus, the method of the invention neither results in significant aggregation of the immunoglobulin (Ig), nor in significant denaturation of the immunoglobulin (Ig). As will be known to the skilled person, the activity of immunoglobulin (Ig) can be determined by standard immunological methods, such as ELISA, flow cytometry and cell-based assays.

When comparing the IgG activity and/or integrity of the composition of the invention pre- and post-nebulization, no damage to IgG due to shear forces and potential temperature in-crease due to vibration of the membrane of a vibrating mesh nebulizer was found. Thus, by using serine as a stabilizer according to present invention, the Ig activity and/or integrity is maintained during the nebulization process. In particular, all values for aggregates, dimers, monomers, fragments, Fc function, anti-Streptolysin O, anti-Parvovirus 19 and anti-HBS have been demonstrated herein by the inventors to be in acceptable ranges for the composition of the invention pre- and post-nebulization.

According to a preferred embodiment, the nebulizer used in the method disclosed herein is a vibrating mesh nebulizer. Vibrating mesh nebulizers comprise a reservoir in which the liquid for the nebulization is filled. When operating the nebulizer, the liquid is fed to a membrane that is made to oscillate, i.e. vibrate (e.g. by means of a piezoelectric element). The liquid present at one side of the vibrating mesh is hereby transported through openings in the vibrating mesh (also referred to as “pores” or “holes”) and takes the form of an aerosol on the other side of the vibrating mesh. Examples of vibrating mesh nebulizers are AdheResp Vibrating Mesh Nebulizer and Deepro Vibrating Mesh Nebulizer (both HCmed Innovations Co., Ltd., Taiwan), eFlow rapid and eRapid from PARI, HL100 from Health and Life as well as AeronebGo and AeronebSolo from Aerogen. Such nebulizers may also be referred to as “active membrane nebulizers”. In general, the nebulizer should be suitable for generating an aerosol targeting the conducting airways of the subject, in particular generations one to thirteen of the respiratory tract of the subject.

It should be noted, that the advantages of the aerosol formation as described herein is primarily a result of the serine stabilizer and not of the specific construction of the nebulizer.

More particularly, a preferred nebulizer for targeting the conducting airways is a nebulizer adapted for generating an aerosol at an effective flow rate of less than about 5 liters/min and for simultaneously operating means for effecting a pressure pulsation of the aerosol at a frequency in the range from about 10 to about 90 Hz, wherein the effective flow rate is the flow rate of the aerosol as it enters the respiratory system of the patient. Examples of such electronic nebulization devices are disclosed in WO2009/027095.

In a preferred embodiment of the invention, the nebulizer for targeting the conducting airways is a nebulizer which uses a transportation flow that can be interrupted when the aerosol cloud

reaches the desired location and then starts the pulsation of the aerosol cloud, e.g. in an alternating mode. The details are described in WO 2010/097119 A1 and WO 2011/134940 A1.

For administration to the respiratory tract of the subject, the liquid aqueous composition of the invention used to generate the aerosol may in particular be administered in a volume of 1 to 10 mL.

The method of generating an aerosol preferably does not include any process steps of electrospraying or dry particle formation.

The area of the lung that is targeted is the bronchioles and the terminal bronchioles. It is not intended that the aerosol, i.e. the medicament gets in the deep lung because it is known that in this area of the lung absorption out of the lung happens. Hence, according to a preferred embodiment, the generated aerosol is for targeting the conducting airways of a patient, in particular the first through the thirteenth generation of the respiratory tract.

According to a preferred embodiment, the respirable delivered dose of the aerosol is higher compared to a comparative aerosol comprising or essentially consisting of a comparative composition that does not comprise serine, but another stabilizer instead. According to a further preferred embodiment, the respirable delivered dose of the aerosol is higher compared to a comparative aerosol that comprises proline instead of serine.

According to a fourth aspect, the present invention relates to an aerosol comprising particles comprising or essentially consisting of a composition as disclosed herein. The aerosol is obtained or obtainable by a method as disclosed herein.

The aerosol according to the present invention, herein also referred to as serine containing aerosol or aerosol, allows for a targeted, localized administration directly to the respiratory tract, preferably by inhalation via the mouth. Accordingly, this dosage form may require the administration of a smaller dose to achieve the same exposure to immunoglobulin in the respiratory tract as would be obtained by using an aerosol that does not comprise serine, but another stabilizer instead. As a result, direct administration to the respiratory tract may be more cost-effective, because less composition is required to achieve the same therapeutic effect in the respiratory tract. Further, due to the aerosol attributes such as higher percentage of certain particle sizes, a better targeting to the conducting airways, in particular generation one to thirteen of the respiratory tract, of the subject is possible.

Two values can be determined experimentally and may be useful to describe the particle size or droplet size of the generated aerosol, i.e. the mass median diameter (MMD) and the mass median aerodynamic diameter (MMAD). The difference between the two values is that the mass median aerodynamic diameter (MMAD) is normalized to the density of water (equivalent aerodynamic). The mass median diameter (MMD) may be measured by laser diffraction, for example using the Malvern MasterSizer X™. The mass median aerodynamic diameter (MMAD) may be measured by an impactor, for example the Anderson Cascade Impactor (ACI) or the Next Generation Impactor (NGI) following Eur Ph 2.9.44 (equivalent to USP 1601). Specifically, the aerosol is introduced to the Next Generation Impactor (NGI) at a rate of 15 L/min. After a certain time that is dependent on the formulation and nebulizer combination, as you need enough time to get consistent collected mass and not too much time to overload the impactor, the cups that collect the drug are collected from each stage of the impactor to determine the MMAD, FPF and the geometric standard deviation (GSD) as well as the mass mean aerodynamic diameter.

Generally, small airways, which are defined by an internal diameter lower than 2 mm, represent almost 99% of the lung volume and therefore play an important role in lung function. Alveoli are sites in the deep lungs where oxygen and carbon dioxide are exchanged with the blood. Inflammation in the alveoli induced by some viruses or bacteria leads to fluid secretion on site and directly affects oxygen uptake by the lungs.

Optimum lung deposition is achieved with droplets between 3 and 5 µm in size [Kane et al. *Inflamm. Allergy Drug Targets*, **2013**, 12, 81-87]. Because delivery of the aerosol according to the present invention is targeted to the conducting airways, the desired aerodynamic diameter of the aerosol particles is 3 to 5 µm (Byron, *J. Pharm. Sci.*, **1986**, 75, 433- 438; Köbrich et al., *Ann. Occup. Hyg.*, **1994**, 38, 15-23; Wang et al., *Int. J. Pharm.*, **2014**, 461, 112). Aerosol characteristics of the aerosol according to the present invention, including droplet size, are appropriate for targeting the conducting airways. According to a preferred embodiment, the particles comprised in the aerosol have a mass median aerodynamic diameter (MMAD) in the range of from 3 to 5 µm, measured by an impactor. In the event that the determination of the mass median aerodynamic diameter (MMAD) using Anderson Cascade Impactor (ACI) and Next Generation Impactor (NGI) leads to differing results, the determination using Eur Ph 2.9.44 is decisive. Particles having a mass median aerodynamic diameter (MMAD) in the above range are particularly useful to achieve a high local drug concentration in the target region or tissue of the respiratory tract of humans, i.e. the conducting airways, including the bronchi and bronchioles, relative to the amount of drug

which is aerosolized. In particular, particles featuring such mass median aerodynamic diameter are particularly useful to target the first through the thirteenth generation of the respiratory tract.

In aerosol therapy, it is common to evaluate the fraction of droplets, i.e. particles, smaller than 5 µm, representing the fraction that is respirable by an adult. This fraction is also referred to as the "fine particle fraction" or "FPF". According to a preferred embodiment, the aerosol has a fine particle fraction of more than 40%, preferably more than 50%, and most preferably of more than 65%.

According to a further preferred embodiment, the aerosol has a higher fine particle fraction as compared to a comparative aerosol comprising or essentially consisting of a comparative composition that does not comprise serine. In particular, the aerosol may have a higher fine particle fraction as compared to a comparative composition that comprises proline instead of serine.

The FPF of a serine containing aerosol is increased by up to 20%, up to 30%, up to 40%, up to 50%, or up to 60%, in particular, up to 50% depending on the particle size and compared to a respective aerosol containing proline.

The FPF in terms of particles smaller than 5µm of a serine containing aerosol is increased by up to 2%, up to 3%, up to 4%, up to 5%, or up to 10%, in particular, up to 5% compared to a respective aerosol containing proline. According to one embodiment, the FPF (< 5µm) of a serine containing aerosol is increased by 5% compared to a respective aerosol containing proline.

The fine particle dose (FPD) of a serine containing aerosol is increased by up to 10%, up to 15%, up to 20%, up to 25%, or up to 30%, in particular, up to 22% compared to a respective aerosol containing proline. According to one embodiment, the fine particle dose of a serine containing aerosol is increased by 22% compared to a respective aerosol containing proline.

The aerosol or method of the invention can also be characterized by its delivered dose (DD) or delivered dose % (DD%), for example as determined in breath simulation experiments. More specifically, breathing simulation experiments may be conducted using a breathing simulator (e.g. BRS3000 from Copley or Compass II™ from PARI) and an adult breathing pattern according to Ph. Eur. 2.9.44, i.e. sinusoidal flow with a tidal volume of 500 mL, 15 breaths per minute and an inhalation:exhalation (I:E) ratio of 50:50. In each test, a nebulizer is connected to a sinus pump (e.g. PARI Compass II™ breath simulator). An inspiratory filter (e.g. polypropylene, 3M) is installed between the nebulizer including the mouth piece and the pump and fixed with air-tight

connectors. The nebulizer is filled with a clinically relevant quantity of the composition (e.g. 200 mg Ig, 200 mg IgG, 200 mg IgA, 200 mg IgM or combinations thereof) and nebulization is initiated and continued until aerosol production is no longer visible or the nebulizer shuts off. The drug containing aerosol droplets are collected on the inhalation filter. To determine the delivered dose (DD), i.e. the amount of immunoglobulin collected on the filter during nebulization, the filter and the residue on the filter case is selected and extracted. Additionally, the nebulizer is also rinsed to determining the amount of drug remaining in the reservoir. The resulting solutions are then analyzed and the results of these analyses is used for the calculation of the delivered dose (DD). The individual steps can be as follows: the inhalation filter is removed from the filter casing with forceps and is put in a 50 mL plastic tube with a screw cap. Afterwards, the filter casing is rinsed with 50 mL buffer containing 0.9% saline and 0.5% SDS (sodium dodecyl sulphate, 98.5%) in purified water, and the rinsing fluid is subsequently added to the tube with the filter. The filter is extracted for 1 h while shaking on a rotator. Additionally, the nebulizer is rinsed several times with 40 mL of the above described buffer and the rinsing solution is collected in a beaker for determining the amount of drug remaining in the reservoir (residue). The solutions resulting from the filter extraction and from the rinsing of the nebulizer are analyzed using UV spectrophotometry. A sample of each of the solutions is diluted with buffer to arrive at a concentration of approximately 0.5 mg/mL immunoglobulin. Approximately 0.8 mL of the diluted sample solution is filled in a disposable micro cuvette and measured against buffer at 280 nm. The immunoglobulin content in the solution is calculated according to the Lambert-Beer law ($A = \varepsilon \cdot c \cdot L$) using the mass absorption coefficient of ε (0.1%) = 1.38 mL/(mg·cm). More specifically, the formula to calculate the immunoglobulin content is:

c (mg/mL) = dilution factor*A_{280}/ε*L

In accordance with present invention, the delivered dose (DD) can be calculated as follows: If c, for example like shown above, is the concentration of the IgG solution, then
DD (mg) = dispensed volume (mL)* c (mg/mL).

With other words, the delivered dose refers to the amount (mg) of IgG delivered in a method of the invention. An alternative way to describe the DD is that this is the dose that is available to enter the respiratory system (for example Tepper et al 2016).

In accordance with present invention, the delivered dose % (DD%) can be calculated as follows:
DD% = DD (mg)/ Theoretical IgG dose (mg)* 100, wherein
Theoretical IgG dose (mg)= filled quantity of composition (mL)* IgG concentration (mg/mL).

With other words, the DD% is the DD expressed as a percentage of the dose in the reservoir of the nebulizer. For example, if in a breath simulation experiment as described above, 2 mL is filled into the reservoir of a nebulizer and 1 mL is collected in the filter, this equals 50% DD%.

In general, the delivered dose (DD), also referred to herein as respirable delivered dose, of serine-containing composition or aerosol is increased by up to 10%, up to 15%, up to 20%, up to 25%, or up to 30%, in particular, up to 18% compared to a proline-containing composition or aerosol. In one embodiment, the delivered dose of serine-containing composition or aerosol is increased by 18% compared to a proline-containing composition or aerosol.

The delivered dose in percent (DD%) of serine-containing composition or aerosol is increased by up to 10%, up to 15%, up to 20%, up to 25%, or up to 30%, in particular, up to 18% compared to a proline-containing composition or aerosol. In one embodiment, the delivered dose of serine-containing composition or aerosol is increased by 18% compared to a proline-containing composition or aerosol.

According to a further preferred embodiment, the total output rate (TOR) for a serine-containing composition or aerosol, when nebulized and/or delivered by a method as described herein, is at least 385 mg/min delivered amount of composition, for example of the liquid formulation, at least 385 mg/min, at least 390 mg/min, at least 395 mg/min, at least 400 mg/min, at least 405 mg/min, or at least 410 mg/min. The TOR is in particular between 385 mg/min and 410 mg/min delivered amount of composition or aerosol.

The TOR is a measure of how much, i.e. the mass, of the composition (mg) is being nebulized per minute during nebulization. More specifically, the output is the mass of aerosol emitted by the nebulizer device at the aerosol outlet port for the given filled quantity of lg composition, measured by initial weight of the device with the lg composition minus the final weight of the device post nebulization, with the residual lg composition. The total output rate is provided as output per unit of time, usually expressed in mg/min.

According to a further preferred embodiment, the total output rate (TOR) of a serine-containing composition or aerosol, when provided by a method as described herein, is increased by about 5% or 10%, in particular, by about 7%; when compared with a composition or aerosol not containing serine or containing proline instead.

According to a fifth aspect, the present invention relates to the use of serine, in particular L-serine, for increasing at least one of the immunoglobulin (Ig) long-term stability, proportion of the fine particle fraction (FPF), fine particle dose (FPD), total output rate (TOR), delivered dose (DD), delivered dose percent (DD%) and palatability of a composition as disclosed herein or of an aerosol as disclosed herein compared to a comparative composition or aerosol that does not comprise serine. The respective favorable effects of serine on the respective parameters have been described herein and demonstrated by the examples.

In particular, the present invention relates to the use of serine, in particular L-serine, for increasing at least one of the immunoglobulin (Ig) long-term stability, proportion of the fine particle fraction (FPF), fine particle dose (FPD), total output rate (TOR), delivered dose percent (DD%) and palatability of a composition as disclosed herein or of an aerosol as disclosed herein compared to a comparative composition or aerosol that comprises proline instead of serine.

The term “about” in relation to a numerical value x is optional and means, for example, any number within 1%, 5% or 10% of the referenced number. The term “about” also encompasses the exact number recited.

Throughout this specification the word “comprise”, or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Furthermore, as used herein the singular forms of “a”, “and” and “the” include plural references unless the context clearly dictates otherwise.

The following examples serve to further illustrate the invention. The examples are not to be understood as limiting.

Brief description of the drawings

Figure 1: Nebulized IgG formulation screening data (melting temperature, 50% unfolded);

Figure 2: Nebulized IgG formulation screening data (onset of unfolding);

Figure 3A: Summarized results of accelerated stability program (absorbance);

Figure 3B: Summarized results of accelerated stability program (turbidity);

Figure 4A: Summarized results of accelerated stability program (molecular size distribution; monomers and dimers);

Figure 4B: Summarized results of accelerated stability program (molecular size distribution; dimers);

Figure 4C: Summarized results of accelerated stability program (molecular size distribution; fragments);

Figure 4D: Summarized results of accelerated stability program (molecular size distribution; aggregates);

Figure 5: Aerosol characteristics of 7% IgG L-proline vs. 7% IgG L-serine;

Figure 6: Total output rate of 7% IgG L-proline vs. 7% IgG L-serine.

Example 1: Formulation screening and a comparative study of serine-based formulation and proline-based formulation

The thermal stability of the IgG protein in solutions was investigated by assessing the melting temperature T_m at which 50% of the protein is unfolded. This provides a good idea on how the protein will behave in the longer term in a stability program. As the formulation to be nebulized was considered, in the nebulized space, to be a concentrated drug, the inventors looked for excipients that could potentially reduce viscosity. Also, the excipients should be “sweet tasting” or taste neutralizing according to the literature. Finally, the excipient should have the potential to maintain stability of the protein. Based on the aforementioned attributes, the following excipients were selected as a starting point for the first screening experiment: Glycine, Serine, Arginine-HCl, Guanidine, Proline, Erythritol, Lactose, Mannitol, Sorbitol, and combinations thereof. Hence, more than 40 formulations with single excipients or combinations of excipients and varying IgG-concentrations were prepared and evaluated for key target parameters including viscosity conductive to nebulization (2.3 to 3 mPa·s), pH conducive to lung and/or nebulization (pH 4.5 to 5.0), osmolality close to an isotonic formulation to avoid inducing any adverse reaction in the lung such as coughing reflexes (150 to 549 mOsmol/kg), aerodynamic diameter for deposition to conducting airways (3 to 5 µm), sweet tasting/palatability, chemical stability (thermal stability, turbidity), long term stability (SE-HPLC, Fc function), and ease of manufacture.

Figure 1 shows an overview of some of the immunoglobulin formulations that were examined in the course of early formulation screening studies. Thermal screening techniques were used to determine the temperature T_m at which 50% of the immunoglobulin (Ig) is unfolded. The formulations contained immunoglobulin G (IgG) either at a concentration of 7% (70 mg/mL) or 10% (100 mg/mL) and excipients at varying concentrations. From a stability standpoint, clusters of several

7% formulations were identified that looked promising from a stability standpoint and even aerosol perspective. However, other attributes such as solubility of the excipients used, their sensation upon nebulization, and/or ease of manufacture have excluded their further investigation. However, it was surprisingly found that, compared to the formulations containing proline, the melting temperature T_m is higher for formulations prepared with serine, i.e. it was found that serine has a protective effect on the unfolding of the protein. Formulations "Ig10 / Pro250 (Privigen)" (denoted by reference sign "1") and "Ig10 / Ser250" (denoted by reference sign "4") are direct comparators as are "Ig7 / Pro175 (diluted Privigen)" (denoted by reference sign "2") and "Ig7 / Ser150" (denoted by reference sign "5"). As can be seen from the data presented in Figure 1, formulations 3, 4 and 5 containing L-serine have a higher T_m than those containing L-proline, i.e. formulations 1 and 2, for both 7% and 10% immunoglobulin concentrations, inferring enhanced stability of the protein. By contrast, sugar alcohols such as erythritol, lactose, mannitol and sorbitol, which would have been the primary choice in terms of "sweet tasting" of the corresponding formulations, have not proved to be beneficial excipients. In particular, mannitol, which has already been approved for pulmonary delivery, was found to induce cough in the patient (S. Spector, Use of Mannitol Inhalation Challenge in Assessment of Cough, Lung (2010), 188(Suppl 1):S99–S103). Similarly, erythritol and sorbitol were either not stabilizing or showed unfavorable nebulization because their use led to an increase in viscosity. The remaining formulations whose T_m lies in the gray area shown in Figure 1, which extends above 54°C, also showed a protective effect on the unfolding of the protein but were ruled out for other reasons later in the screening.

The pre-screening process described above is noteworthy because the inventors of the present invention started with a vast number of excipient candidates and combinations thereof and ended up with a single compound. In particular, there is an element of surprise that, although serine could potentially be stabilizing, at high concentrations of IgG serine can be both stabilizing and viscosity reducing on its own, i.e. without combination of other amino acids or sugars.

Figure 2 shows the onset of unfolding of the protein T_{on}, which was again investigated using thermal screening techniques. With formulation 1, the temperature at which the protein starts to unfold was found to be around 53°C, which is relatively low. By contrast, the comparable serine formulation 3 featured a temperature at which onset of unfolding occurs of around 55°C. Similarly, the formulation 2 containing 7% L-proline features a temperature at which onset of unfolding occurs of around 53°C whereas the comparable formulations 4 and 5 comprising L-serine featured a significantly higher temperature of around 55°C. Again, it was concluded that serine appears to have a protective effect on the conformational stability of the immunoglobulin (Ig) protein comprised in the tested compositions.

Based on the results described and shown in Figures 1 and 2, the candidates located in the gray area extending above 54°C in Figures 1 and 2 were further investigated in a longer-term stability program because these formulations showed a beneficial effect in terms of thermal stabilization of the protein. Stability program was carried out according to the general principals of the International Conference of Harmonisation (ICH); ICHQ1(R2)- Stability testing of new drug substances and products. The formulations were stored at various temperature conditions of 2-8°C, 25°C/60%RH and 37°C/60%RH 2-8°C, at relative humidity, and at relative humidity over a period of up to 6 months. Figure 3A shows the measured absorbance in the wavelength range from 350 nm to 500 nm for the formulations "7% IgG 175 mM Proline" (denoted with reference sign "2"), "10% IgG 250 mM Proline" (denoted with reference sign "1"), "7% IgG 250 mM Serine" (denoted with reference sign "4"), "10% IgG 250 mM Serine" (denoted with reference sign "3", and "7% IgG 270 mM Serine" (denoted with reference sign "6"), in each case at the initial time T0 (A), after 3 months at 2-8°C (B), after 6 months at 2-8°C (C), after 3 months at 25°C (D), after 6 months at 25°C (E), after 3 months at 37°C (F) and after 6 months at 37°C (G). Similarly, Figure 3B shows the measured turbidity, determined in units NTU, for the aforementioned formulations 1-4 and 6 against the elapsed time A-G. Formulations 1 and 3 are comparable as are formulations 2 and 6. No significant differences in the data were observed for absorbance and turbidity, which indicates that the compositions containing serine instead of proline have a comparable long-term stability as proline stabilized 10% (w/v) IgG.

Figures 4A-4D show the molecular size distribution observed for aqueous immunoglobulin (Ig) compositions 1, 2, 3, 4 and 6, in each case at the initial time T0 (A) and when stored at 2-8°C for 3 months (B), when stored at 2-8°C for 6 months (C), when stored at 25°C for 3 months (D), when stored at 25°C for 6 months (E), when stored at 37°C for 3 months (F) and when stored at 37°C for six months (G). Apart from a small demonstrated increase in dimers in the 10% IgG 250 mM L-serine formulation when stored at 2-8°C, i.e. formulation 3 under conditions B and C, compared to others, no significant differences were observable in the data. In any case, this small increase in the formation of dimers has no impact on the stability of the immunoglobulin (Ig) composition.

In order to compare the tested formulations in terms of nebulization performance, nebulization experiments were conducted with formulations "7% IgG L-serine" (denoted with reference sign 8) and "7% IgG L-proline" (denoted with reference sign 7) by measuring the following characteristics: Delivered Dose (DD), mass median aerodynamic diameter (MMAD), fine particle fraction and dose (FPF, FPD), total output rate (TOR), i.e. how much is being delivered per minute of delivery. The devices used in the nebulization studies were of the vibrating mesh type nebulizer and

operated in continuous mode, in particular a Deepro Vibrating Mesh Nebulizer (HCmed Innovations Co., Ltd., Taiwan) was used. Two analytical tools are used that are both compendial methods. On the one hand, next generation impactor (NGI) following Eu guidance 4.2.1.8. On the other hand, delivered dose and total output rate (TOR) were measured utilizing a breath simulator with a 1:1 (inhalation to exhalation rate) tidal sine wave, 15 breaths per minute profile compliant with EU PH chapter 2.9.44. Time to deliver an entire dose volume was also measured and recorded.

For the nebulization experiments, a next generation impactor (NGI) device was turned on and the respective composition was continuously expelled from the device using a vibrating mesh. The composition is pushed in the device's collection area comprising multiple teardrop-shaped wells each acting as collection chamber. The aerosols are delivered in a polydispersion of particles and particle sizes. Each of the particle sizes is captured by one of the wells and the immunoglobulin is trapped on the respective filters in the wells. Using this device, the delivered amount of immunoglobulin in each of the droplet size across the distribution can be measured. For the breath simulator, the device was attached to an artificial lung and then the breathing pattern of inhalation and exhalation occurred in a 1:1 fashion (inhalation to exhalation rate 1:1). The time to nebulize was recorded and the delivered dose (DD) was also recorded.

The data shown in Figure 5 indicates that the delivered dose (DD), all given fine particle fractions (FPF) as well as the fine particle dose (FPD) are all higher for the 7% IgG L-serine formulation 8 compared to the 7% IgG L-proline formulation 7. The DD was improved by approximately 18%, the FPF (< 5µm) by approximately 5%, the FPF (< 3µm) by approximately 11%, the FPF (< 1µm) by approximately 50% and the FPD by approximately 22% based on the determined mean values.

For figure 5, several tests on different nebulizing devices were conducted, with 7% serine-based formulation and 7% proline-based formulation. The overall corresponding raw data resulting from these comparative studies are presented in tables 1 and 2, respectively. The tests were conducted with 6 and 5 different devices of the Deepro Nebulizer type, respectively.

Table 1 – 7% serine-based formulation

	Device	**1**	**2**	**3**	**4**	**5**	**6**	**TOTAL**
Delivered dose in mg	Mean	109.11	132.15	130.71	124.96	131.15	129.21	124.51
	Std Dev	5.79		11.14	2.44	1.75	7.04	10.57
	Min	104.05	132.15	119.86	123.50	129.60	121.16	104.05
	Max	116.16	132.15	142.12	127.78	133.05	134.20	142.12
	N	4	1	3	3	3	3	17
FPD<5µm µg/dose	Mean	52.16	45.67	59.95	53.38	48.43	62.71	54.57
	Std Dev	3.38		5.53	1.16	0.94	16.41	8.46
	Min	49.07	45.67	54.46	52.16	47.38	49.76	45.67
	Max	56.84	45.67	65.52	54.46	49.19	81.17	81.17
	N	4	1	3	3	3	3	17
FPF<5µm % of Sum	Mean	47.79	34.56	45.84	42.72	36.94	48.35	43.96
	Std Dev	1.09		0.36	0.57	1.15	11.29	6.28
	Min	46.85	34.56	45.43	42.20	35.61	41.07	34.56
	Max	48.93	34.56	46.10	43.33	37.61	61.36	61.36
	N	4	1	3	3	3	3	17
FPF<3µm % of Sum	Mean	25.06	16.54	24.51	21.59	18.09	27.37	23.03
	Std Dev	1.52		1.56	0.62	0.87	11.34	5.43
	Min	23.63	16.54	22.83	20.91	17.12	20.42	16.54
	Max	27.06	16.54	25.93	22.13	18.80	40.46	40.46
	N	4	1	3	3	3	3	17
FPF<1µm % of Sum	Mean	1.40	0.59	1.52	1.02	0.77	2.33	1.36
	Std Dev	0.77		0.90	0.18	0.25	2.13	1.05
	Min	0.82	0.59	0.96	0.83	0.51	1.06	0.51
	Max	2.52	0.59	2.56	1.19	1.01	4.79	4.79
	N	4	1	3	3	3	3	17
MMAD µm	Mean	4.60	5.68	4.72	4.97	5.40	4.50	4.87
	Std Dev	0.12		0.12	0.05	0.12	0.91	0.50
	Min	4.44	5.68	4.64	4.92	5.31	3.44	3.44
	Max	4.70	5.68	4.86	5.01	5.53	5.06	5.68
	N	4	1	3	3	3	3	17
GSD	Mean	2.00	2.10	2.07	2.04	2.06	2.04	2.04
	Std Dev	0.03		0.03	0.03	0.01	0.04	0.04
	Min	1.98	2.10	2.04	2.00	2.05	2.02	1.98
	Max	2.04	2.10	2.10	2.05	2.07	2.08	2.10
	N	4	1	3	3	3	3	17

Table 2 – 7% proline-based formulation

	Device	**1**	**2**	**3**	**4**	**5**	**TOTAL**
Delivered dose	Mean	102.37	103.57	102.59	102.93	101.96	102.68
	Std Dev	9.06	6.20	9.08	8.27	2.66	6.33
	Min	94.65	98.28	92.56	94.69	98.89	92.56
	Max	112.35	110.39	110.23	111.22	103.61	112.35
	N	3	3	3	3	3	15
FPD<5µm µg/dose	Mean	45.30	43.15	41.38	42.84	41.46	42.83
	Std Dev	5.56	5.04	5.36	4.72	1.51	4.22
	Min	40.71	39.12	36.04	38.08	39.85	36.04
	Max	51.49	48.79	46.75	47.53	42.84	51.49
	N	3	3	3	3	3	15
FPF<5µm % of Sum	Mean	44.16	41.57	40.24	41.56	40.66	41.64
	Std Dev	1.48	2.33	1.89	1.27	0.68	1.97
	Min	43.01	39.80	38.94	40.22	40.24	38.94
	Max	45.83	44.20	42.41	42.73	41.44	45.83
	N	3	3	3	3	3	15
FPF<3µm % of Sum	Mean	21.88	20.68	19.71	20.55	19.49	20.46
	Std Dev	2.02	2.37	2.73	2.24	1.09	2.03
	Min	20.04	18.51	17.25	18.12	18.84	17.25
	Max	24.04	23.21	22.64	22.54	20.76	24.04
	N	3	3	3	3	3	15
FPF<1µm % of Sum	Mean	0.65	0.69	1.03	0.69	0.37	0.68
	Std Dev	0.72	0.85	1.38	0.74	0.41	0.77
	Min	0.04	0.04	0.03	0.03	0.10	0.03
	Max	1.44	1.65	2.60	1.50	0.83	2.60
	N	3	3	3	3	3	15
MMAD µm	Mean	4.81	5.00	5.12	5.02	5.07	5.00
	Std Dev	0.16	0.20	0.24	0.19	0.11	0.19
	Min	4.63	4.80	4.86	4.84	4.96	4.63
	Max	4.93	5.19	5.32	5.21	5.18	5.32
	N	3	3	3	3	3	15
GSD	Mean	2.03	2.06	2.08	2.06	2.04	2.05
	Std Dev	0.05	0.06	0.08	0.07	0.02	0.05
	Min	1.98	2.01	2.00	1.99	2.02	1.98
	Max	2.08	2.13	2.15	2.12	2.05	2.15
	N	3	3	3	3	3	15

Additionally, as can be seen from the breath simulation studies summarized in Figure 6, the L-serine formulation 8 has a much higher total output rate (409 mg/min) compared to the L-proline formulation 7 (383 mg/min). This is an increase of about 7%. The L-serine formulation appears to be delivered more efficiently, i.e. with a greater output. The output is what has left the device, measured by initial weight of the device with the drug minus the final weight of the device post nebulization, with the residual drug. The total output rate is provided as output/time to nebulize in mg/min. Similarly, the total delivered dose was found to be higher and able to be delivered with a higher output rate for the 10% IgG L-serine formulations (120 mg/min) compared to the analogue formulations stabilized with L-proline (100 mg/min, data not shown). This demonstrates that serine is able to enhance nebulizeability, independent of protein concentration. Apart from that, mass median aerodynamic diameters (MMAD) and geometric standard deviations (GSD) of the respective comparable compositions containing 7% and 10% immunoglobulin G (IgG) were equivalent.

With a further set of experiments it was investigated whether there is any potential damage to IgG due to shear forces and potential temperature increase due to vibration of the membrane of a vibrating mesh nebulizer. The drug integrity pre- and post- nebulization for a nebulizer device, here an AdheResp device (HCmed, Taiwan), in combination with the 7% IgG serine formulation of the invention and a proline formulation as reference was tested.

Per each run, the medication container of the device was filled with 8 mL 7% IgG formulation using a glass pipette. The results are based on three AdheResp devices from one assembly lot and one serine based 7% IgG lot. One lot of 10% proline-based IgG formulation diluted to 7% IgG formulation was used as control. To collect the required amount of aerosol for the analytical assessment, the device went through several rounds of nebulization. The recovered liquid of each device and corresponding runs were polled in a separate 50 mL Falcon tubes.

For collecting the aerosol, a 50 mL Falcon tube was covered with a silicone seal that included a hole. The container lid of the medication container was covered with Parafilm to prevent aerosol from escaping. The aerosol port was inserted into the hole of the silicone seal of the Falcon tube. This design minimized the loss of aerosol and enabled the condensation of the aerosol within the Falcon tube. The recovered liquids (post-nebulization) of each device and the corresponding runs were pooled in separate 50 mL Falcon tubes. Immediately after completion of the test series, the pooled liquid in the 50 mL Falcon tubes were aliquoted into 15 mL Falcon tubes. The pre-nebulized formulations were filled into 15 mL Falcon tubes.

The following quality attributes were evaluated for their relevance to demonstrate drug integrity pre- and post-nebulization:

Molecular size distribution (Aggregates, Monomers, Dimers, Fragments); Proline, respectively Serine, content; Fc function; Anti-Streptolysin O-antibodies; Anti-HBs-antibodies; Anti-Parvovirus B-19; pH; Osmolality; and protein content. Quality attribute “Fc function” and antibody-specific tests – anti-HBs, anti-Streptolysin O and anti-Parvovirus B-19- were measured to ensure the product potency and for the functional integrity of the Fc and Fab region of the IgG molecule. The antibody-specific parameters monitor the potency as representative for IgG molecules against viruses and bacteria, respectively.

As a result, the acceptance criteria for all mentioned quality attributes are fulfilled for the tested proline- as well as serine-based IgG formulations. The drug integrity is not affected based on the results.

It can be concluded that there are no relevant changes of the IgG composition characteristics due to the shear forces and temperature increase caused by the vibration of the mesh during nebulization. The nebulization process has no effect on drug integrity of a serine stabilized IgG solution. This again demonstrates that serine is a suitable stabilizer in the composition of present invention, in particular suitable for nebulization as described herein.

It is important to note that in the conducted experiments, serine was not the most stabilizing excipient but taken together with its other attributes, it was found to be the best candidate as it provided both good protein stabilization and performed superior in terms of aerosol generation and aerosol characteristics with regard to suitability for inhalation.

Example 2: Toxicity studies of nebulized L-serine/IgG (Safety)

A study was conducted with cynomolgus monkeys to determine the potential toxicity of human normal immunoglobulin (IgG) when administered by nebulization. One of the objectives of this study was to determine whether L-serine and/or IgG could be safely delivered to the lungs by nebulization. The local and systemic bioavailability of IgG was also analysed.

a) Study design

A 7% formulation of human plasma-derived polyvalent immunoglobulin G (IgG) was administered to cynomolgus monkeys *via* nebulization. This formulation contained a protein concentration (IgG) of 70 mg/ mL and 270 mmol/L L-serine (stabilizer) having a pH of 4.6 – 5.0 and Osmolality of 280 – 310 mOsmol/kg.

Three doses of IgG were tested – (1) a low dose (5 mg/kg/day); (2) an intermediate dose (13 mg/kg/day) and a high dose (24 mg/kg/day). Two controls were used – an air control and a L-serine vehicle control. A summary of the study design is provided in the table 3 below:

Table 3 – Study design

Treatment Group	**Phase**	**Number of Animals**	
		Males	**Females**
Air Control	26 Week Main Study	3	3
Vehicle Control	4 Week Main Study	3	3
	26 Week Main Study	3	3
	13 Week Recovery	3	3
Low Dose	26 Week Main Study	3	3
Intermediate Dose	26 Week Main Study	3	3
High Dose	4 Week Main Study	3	3
	26 Week Main Study	3	3
	13 Week Recovery	3	3

The following parameters and end points were evaluated: mortality, clinical observations, body weights, respiratory measurements, electrocardiology, neurological examinations, clinical pathology parameters (haematology, coagulation, clinical chemistry, and blood and BAL urea nitrogen), biomarkers complement analysis (plasma and BAL), bioanalysis, anti-drug antibody analysis, bronchoalveolar lavage cell counts, organ weights and macroscopic and microscopic examinations.

b) Results

IgG administered by nebulization had no effect on body weight, clinical signs, electrocardiography, respiratory measurements, haematology, coagulation, clinical chemistry, plasma biomarker (complement C3a and sC5b-9), BAL biomarker (complement C3a), BAL cell counts, macroscopic pathology or organ weights in cynomolgus monkeys at any of the doses tested.

One BAL biomarker (sC5b-9) was elevated (mild to moderate) at all doses of the 7% IgG formulation (males and females). This finding was considered to be non-adverse. There were no BAL biomarker (sC5b-9) findings which were considered to be related to the daily administration of L-Serine.Immunogenicity analysis revealed a positive immune response in all the animals that received IgG. No toxicological importance was attached to this finding as NHPs often develop an immune response to human derived proteins.

Bioanalysis of plasma samples did not differentiate between monkey and human IgG but IgG levels did not deviate measurably from endogenous baseline values. Suggesting that any therapeutic effects of inhaled IgG will be localised in the lungs rather than systemic.

Bioanalysis of BAL samples at necropsy showed marginal or slight elevation in adjusted BAL IgG values at 12.6 and 24.0 mg IgG/kg/day, without evidence of bioaccumulation.

Following histopathological evaluation of the lungs, non-adverse perivascular/peribronchiolar mononuclear cell infiltration, peribronchiolar macrophage aggregates and increased cellularity of bronchial-associated lymphoid tissue (BALT) were generally observed in both sexes after 4 weeks at 22.7 mg IgG/kg/day and 26 weeks of dosing at all dose levels. There was recovery from these following a 13-week recovery period.

In addition, diffusely distributed alveolar macrophages were observed histopathologically at all timepoints in single males and females exposed to the vehicle L-Serine and therefore was considered a possible effect of vehicle administration. All instances of this finding were of a minimal grade so were considered non-adverse. An additional influence of the 7% IgG formulation was considered equivocal, as this finding was present at a higher incidence at 22.7 mg IgG/kg/day at the 4-week time point only.

The estimated delivered doses of IgG and L-Serine are summarized in Tables 4 and 5. No IgG or L-serine was measured in any in the air control sample.

Table 4 - Estimated Delivered Doses of IgG – Group Mean Values

Dose Group/ Treatment	Estimated Delivered Doses of IgG (mg/kg/day)					Difference from Target (± %)
	Target	Males	Females	Com-bined	%RSD	
26 Week Main and Recovery Study						
Low Dose	5	4.79	4.78	4.78	29.0	-4.3
Intermediate Dose	15	12.3	12.8	12.6	13.5	-16.2
High Dose	30	23.5	24.4	24.0	17.0	-20.1
4 Week Main Study						
High Dose	30	22.1	23.4	22.7	14.6	-24.2

Table 5 - Estimated Delivered Doses of L-serine – Group Mean Values

5

Dose Group/ Treatment	Estimated Delivered Doses of L-Serine (mg/kg/day)			
	Males	Females	Combined	%RSD
26 Week Main and Recovery Study				
Vehicle Control	12.6	12.7	12.6	21.4
High Dose	10.0	10.4	10.2	17.8
4 Week Main Study				
Vehicle Control	9.57	10.0	9.80	21.4
High Dose	9.35	9.94	9.64	16.4

The estimated particle distribution size of the aerosols is summarized in the Table 6 below.

Table 6 - Aerodynamic Particle Size Distribution

Dose Group/ Treatment	MMAD (µm) and GSD	
	Gravimetric	Analytical
Vehicle Control	1.67 (2.271) – 3.47 (2.873)	1.55 (2.516) – 3.67 (2.005)
Low Dose	1.54 (2.214) – 2.82 (1.690)	1.82 (1.354) – 3.13 (1.312)
Intermediate Dose	1.76 (3.442) – 3.08 (1.627)	1.71 (2.415) – 2.99 (1.300)
High Dose	1.89 (2.800) – 4.34 (4.460)	2.17 (1.809) – 3.26 (1.317)

In general, the aerosol particle size distribution was within target ranges (MMAD 2-4 µm). Excursions outside this range were considered not to impact respirability in this model of the delivered aerosols. For each group, delivered aerosols with L-Serine and/or IgG were considered to be respirable to monkeys.

c) Summary

Test item-related findings in the lungs were typical of those reported previously with inhaled biologics (Hall *et al.* (2021) *Toxicologic Pathology,* 49(2):232-234).

Inhalation administration of the 7% formulation of human plasma-derived polyvalent immunoglobulin G (IgG), stabilized with serine as described under a) above, once daily for 26 weeks was well tolerated in monkeys at up to 24.0 mg IgG/kg/day. Based on the target organ (lung) results, the no-observed-adverse effect level (NOAEL) was considered to be 24.0 mg IgG/kg/day.

In addition, inhalation administration of the vehicle L-Serine once daily for 26 weeks was well tolerated in monkeys at up to 12.6 mg L-Serine/kg/day. Based on the target organ (lung) results, the no-observed-adverse-effect level (NOAEL) was considered to be 12.6 mg L Serine/kg/day.

These data therefore demonstrate that nebulized IgG has a positive safety profile in cynomolgus monkeys. In addition, these data demonstrate that L-serine has a favourable safety profile alone and in combination with IgG. Therefore, this example demonstrates that L-serine can be useful as a stabilizer in compositions comprising IgG that are administered by nebulization.

Claims

1. A liquid aqueous composition comprising a polyclonal immunoglobulin (Ig) and a stabilizer, wherein the stabilizer comprises serine, in particular L-serine.

2. The composition of claim 1, wherein the immunoglobulin (Ig) is selected from at least one of polyclonal immunoglobulin G (IgG), polyclonal immunoglobulin A (IgA), and polyclonal immunoglobulin M (IgM).

3. The composition of claim 1 or 2, wherein the immunoglobulin (Ig) is human plasma-derived polyclonal immunoglobulin G (hIgG).

4. The composition of any one of the preceding claims, wherein the stabilizer essentially consists of serine.

5. The composition of any one of the preceding claims, wherein the composition is essentially free of amino acids other than serine.

6. The composition of any one of the preceding claims, wherein the concentration of the immunoglobulin (Ig) is equal to or lower than about 120 mg/mL, preferably in the range of 50 mg/mL to 120 mg/mL, more preferably 60 mg/mL to 110 mg/ mL, even more preferably 60 mg/mL to 100 mg/ mL, further preferably 60 mg/mL to 90 mg/mL, even further preferably 60 mg/ mL to 80 mg/mL and most preferably about 70 mg/mL.

7. The composition of any one of the preceding claims, wherein the concentration of serine is in the range of 100 to 400 mM, preferably 250 to 300 mM, and most preferably about 270 mM.

8. The composition of any one of the preceding claims, wherein the aqueous composition has a pH in the range of from 4.0 to 6.0, preferably 4.5 to 5.0, most preferably a pH of about 4.8.

9. The composition of any one of the preceding claims, wherein the composition has a viscosity in the range of from 2.3 to 3.0 mPa·s, determined by a falling sphere viscosimeter according to Höppler in accordance with the European Pharmacopoeia Version 6.0 at 2.2.49 and the requirements of DIN 53015 at a temperature of 20.0°C +/- 0.1 °C.

10. A method of generating an aerosol, said method comprising the steps:

a) providing a composition as defined in any one of claims 1 to 9;
b) nebulizing the composition provided in step a) by means of a nebulizer, in particular a vibrating membrane nebulizer, thereby generating the aerosol.

11. The method of claim 10, wherein the generated aerosol is for targeting the conducting airways of a patient, in particular the 1st through the 16th generation, or preferably the 1st through the 13th generation of the respiratory tract.

12. The method of claim 10 or 11, wherein at least one of the respirable delivered dose (DD), the delivered dose percent (DD%), the total output rate (TOR), the fine particle dose (FPD), or the proportion of a fine particle fraction (FPF) of the aerosol is higher compared to a comparative aerosol comprising or essentially consisting of a comparative composition that does not comprise serine, in particular a comparative composition that comprises proline instead of serine.

13. An aerosol comprising particles comprising or essentially consisting of the composition according to any one of claims 1 to 9, wherein the aerosol is obtained by the method according to any one of claims 10 to 12.

14. The aerosol of claim 13, wherein the aerosol has a higher respirable delivered dose (DD), a higher delivered dose percent (DD%), a higher total output rate (TOR), a higher fine particle dose (FPD), and/or a higher proportion of a fine particle fraction (FPF) as compared to a comparative aerosol comprising or essentially consisting of a comparative composition that does not comprise serine, in particular a comparative composition that comprises proline instead of serine.

15. Use of serine, in particular L-serine, for increasing

- the immunoglobulin (Ig) long-term stability; and/or
- the proportion of the fine particle fraction (FPF); and/or
- fine particle dose (FPD); and/or
- the total output rate (TOR); and/or
- the delivered dose (DD); and/or
- delivered dose percent (DD%) and/or
- the palatability

of a composition according to any one of claims 1 to 9 or of an aerosol according to any one of claims 13 or 14 compared to a comparative composition or aerosol that does not comprise serine, in particular when compared to a comparative composition or aerosol that comprises proline instead of serine.