Abstract: The invention concerns the field of biomolecule formulations. It concerns a liquid formulation of therapeutic antibodies at high concentrations for pharmaceutical use including the purpose of storage and administration to patients. Based on this formulation the antibodies are in an aqueous solution that buffers changes in pH in absence of a buffer excipient.
BUFFER CAPACITY OF ANTIBODIES

BACKGROUND OF THE INVENTION

TECHNICAL FIELD

The invention concerns the field of biomolecule formulations. It concerns a composition, preferably a liquid formulation, of a therapeutic protein, especially an antibody, at high concentration for pharmaceutical use including the purpose of storage and administration to patients. Further, the invention concerns a method of administration of a liquid formulation of a therapeutic protein, especially an antibody, to a patient, wherein the formulation to be administered to the patient is obtained by diluting a composition which contains the protein at a concentration of at least 100 mg/ml into a larger volume of a solution.

BACKGROUND

Over the past two decades monoclonal antibodies (mAbs) took over a leading role in the biopharmaceutics sector. Numerous mAbs are in the pipelines of biotech and pharmaceutical companies nowadays and over 20 antibody drugs are already on the market (Reichert 2011). The increasing interest in the development of monoclonal antibodies as therapeutics is motivated by the following major advantages. First of all, antibodies inherently possess the potential to act in a very specific manner. Also, they can be used as shuttles for conjugated drug-molecules by which the drug is more efficiently delivered to a specific target. Therefore, high potency and efficacy are attributed to antibody-based therapeutics and they are generally expected to cause fewer side effects than small molecular drugs (Wang 2007).

However, monoclonal antibodies are complex molecules that are susceptible to a variety of degradation routes. Chemical degradation damages the amino acid building blocks and the backbone of the protein molecules affecting the protein primary and secondary structure. In addition, physico-chemical processes can impair the tertiary structure whose integrity is crucial for the biologically active state. Biopharmaceutical development therefore needs to address the degradation issue and seek maximal stability of the antibody therapeutic. A further challenge lies in finding the correct application form of the therapeutic. Due to their proteinogenic origin, mAbs suffer from poor bioavailability when administered via the oral route. Therefore, antibody drug products for parenteral administration are developed. While a
certain dosing needs to be accomplished here, the volume of injected product, especially for subcutaneous applications, is limited by medical compliance issues (injection volume not exceeding 1.5 ml, Guptal999). Therefore, to reach the clinical therapeutic dose, antibody formulations containing highly concentrated protein (up to more than 100 mg/ml) are strongly needed. In addition to the advantages of subcutaneous (sc) application for the patients, the high concentration formulation decreases storage and material cost. If self-administration can be considered, also costs for residence in hospitals and hospital personnel costs are reduced. Chemical degradation of proteinogenic drugs typically includes deamidation, oxidation, proteolysis, β-elimination and cleavage of disulphide bonds. Being strongly pH dependent, these degradation pathways can be controlled by choosing a formulation buffering at a specific pH. Studies on model peptides (Patel 1990, Bhatt 1990, Oliyai 1993, Oliyai 1994) and antibodies (for examples see Wang 2007) revealed that low degradation rates and highest overall stability are generally observed in the range of pH 4.0 to pH 7.5. Also, physico-chemical properties, i.e., colloidal and conformational stability depend on the pH value of the antibody solution. Unlike for the chemical stability, the optimal pH value for ensuring maximal physico-chemical stability varies over the whole pH range and is very specific for a certain mAb molecule. Thus, for solution formulation of a desired therapeutic antibody (e.g. an aqueous solution for parenteral administration), there is a need to select the acceptable pH range to balance the chemical stability, physical stability, thermal stability, and solubility properties of the desired antibody therapeutic (see Rathore AS and Mhatre R 2011). In the case of a mAb molecule it is particularly important to identify the type of chemical modifications and associated molecular regions to aid in the optimization and selection of the solution formulation. For example, forced degradation studies on a particular antibody molecule under development may indicate that the variable region is susceptible to aggregation at acidic pH, oxidation at elevated temperature, and in the presence of trace metals, whereas the constant region is more susceptible to deamidation at alkaline pH region and oxidation at elevated temperature (compare chapter 9.4 in Rathore AS and Mhatre R 2011). The dependence of asparagine deamidation rates in both the Fc and Fab regions of a model mAb on buffer type, pH and temperature was analysed in a recent study by Pace AL et al. (Pace AL 2013), as deamidation of asparagine residues in mAbs can be a major route of
degradation. In general, control of pH is a crucial demand during mAb processing and storage. Conventionally, this is achieved by adding buffering agents (also referred to herein as buffer excipients) such as histidine, citrate and acetate to the antibody solutions. However, buffer type and buffer concentration may also have an effect on protein stability, especially aggregation (Hovgaard L 2012). Asparagine deamidation of a new mAb developed by Eli Lilly was dependent on pH and buffer type, with phosphate buffer formulations having higher deamidation rates than citrate buffer formulations (Zheng and Janis 2006). Therefore, there is a need to provide a method for the solution formulation of a desired therapeutic antibody, which stabilizes the antibody molecule at its optimal pH, and which does not necessitate the addition of buffer excipients to adjust the pH of the solution formulation.

SUMMARY OF THE INVENTION

The invention describes a composition, especially a liquid formulation comprising a pharmaceutical protein, especially an antibody, at high concentration of at least 100mg/ml for pharmaceutical use including the purpose of storage and administration to patients, wherein said pharmaceutical protein/antibody is in an aqueous solution that buffers changes in pH in the absence of a buffer excipient. The invention relates to a method of administrating a composition to a subject comprising the following steps:

a. Providing a composition comprising a protein, preferably an antibody or antibody fragment or Fc-fusion protein, at a concentration of at least 100 mg/ml, wherein said composition does not contain a buffer excipient, having a volume of 0.1 ml to 20 ml, or 1 ml to 10 ml, preferably 1 ml,

b. Diluting said composition of step a) into a larger volume of a solution, wherein the larger volume is between 50 ml to 1 L, or between 100 ml to 500 ml, preferably 250 ml, or 500 ml, or 250 ml to 500 ml,

c. Administrating said solution of step b) to a subject.

In a further aspect, the invention relates to a method of preparing a composition for administration to a subject comprising the following steps:

a. Providing a composition comprising a protein, preferably an antibody or antibody fragment or Fc-fusion protein, at a concentration of at least 100 mg/ml, wherein said
composition does not contain a buffer excipient, having a volume of 0.1 ml to 20 ml, or 1 ml to 10 ml, preferably 1 ml.

b. Diluting said composition of step a) into a larger volume of a solution, wherein the larger volume is between 50 ml to 1 L, or between 100 ml to 500 ml, preferably 250 ml, or 500 ml, or 250 ml to 500 ml.

The invention specifically concerns a (pharmaceutical) composition comprising a (pharmaceutical or preferably therapeutical) protein, preferably an antibody or antibody fragment or Fc-fusion protein, at a concentration of at least 100 mg/ml, wherein said composition does not contain a buffer excipient. Preferably the protein/antibody provides 100% of the buffer capacity of the composition.

In the methods according to the invention a (pharmaceutical) composition comprising a (pharmaceutical or preferably therapeutical) protein, preferably an antibody or antibody fragment or Fc-fusion protein, at a concentration of at least 100 mg/ml is used, wherein said composition does not contain a buffer excipient. Preferably the protein/antibody provides 100% of the buffer capacity of the composition provided in method step a).

In the methods and compositions of the invention the compositions comprising a protein at a concentration of at least 100 mg/ml, wherein said composition does not contain a buffer excipient, may be obtained by reconstitution from a lyophilisate. In the methods of the invention one or more further proteins may be added as a separate composition prior to or subsequently to diluting said composition of step a) into a larger volume of a solution, or as part of the larger volume of a solution used for the dilution of the composition of step a). Said one or more further proteins may be selected from the group consisting of a therapeutic protein, preferably a therapeutic antibody, an antibody fragment, an Fc-fusion protein, a full-length immunoglobulin molecule, an immunoglobulin based molecule such as an immunoglobulin fragment, an immunoglobulin isoform, a fusion protein comprising at least one immunoglobulin chain, or an immunoglobulin conjugated to a non-proteinaceous moiety. Furthermore, the invention provides a kit comprising in one or more containers a composition according to the invention comprising a protein, preferably an antibody or antibody fragment or Fc-fusion protein, at a concentration of at least 100 mg/ml, wherein said composition does not contain a buffer excipient, having a volume of 0.1 ml to 20 ml, or 1 ml to 10 ml,
preferably 1 ml, and a larger volume of a solution according to the invention wherein the larger volume is between 50 ml to 1 L, or between 100 ml to 500 ml, preferably 250 ml, or 500 ml, or 250 ml to 500 ml.

Proteins and antibodies inherently possess a considerable amount of readily ionizable and thus potential buffering groups, i.e., the amino acid side chains of histidine, aspartic acid, glutamic acid, arginine, tyrosine, cysteine and lysine. The side chains of aspartic acid, glutamic acid and histidine exhibit pKa values of 3.7, 4.3 and 6.5, respectively (Thurlkill 2006a) and are therefore capable of providing buffer capacity in the range of pH 3.0 to 7.5 which covers the pH regime of slowest chemical degradation mentioned before. With the upcoming need for highly concentrated mAb solutions used for human application (introduced above), the buffering side chains come in high concentration in the mAb solutions and are able to replace buffer excipients (Gokarn 2008). Actually the role of proteins providing buffer capacity is prevalent in any cellular and intercellular environment (Hainsworth 1986). Besides the bicarbonate system proteins buffer in blood (Bullock 2001) and the buffering in saliva between pH 3.4 and 5 is even primarily accomplished by proteins (Lamanda 2007).

In this patent application we provide a comprehensive characterization of buffer agents widely used in biopharmaceutical manufacturing and antibody formulation. We also determine the buffer capacities of antibodies in high-concentration solutions and their potential to provide self-buffering function. This function could on the one hand be utilized but also might have an impact on the pH of a formulation, if it dominates over buffer excipients.

We derive buffer capacities experimentally in titrations and compare them to ab initio derived values. To allow for calculating buffer capacities of the buffering agent at a certain concentration for practical use, we also determine the concentration dependence of buffer capacity. Since the biopharmaceutical development process also comprises steps where the antibody is kept at different temperatures, we check for the temperature dependence of buffer capacities. Also, the dependence on ionic strength is covered in our study.
Controlling pH values is essential during a variety of steps in the biopharmaceutical process. The chemical stability of biologies such as monoclonal antibodies is pH-dependent and slightly acidic conditions are recommended. Since control of pH is widely provided by buffer salts, the current study summarizes the buffer characteristics of acetate, citrate, histidine, succinate and phosphate buffers under isotonicity conditions. Experimental values largely coincide with values calculated from a model that had already been proposed in 1922 by van Slyke (van Slyke 1922). As high concentrated protein formulations become more and more prevalent, the self-buffering potential of proteins might replace conventionally added buffer salts. The current study shows that a monoclonal antibody at 50 mg/ml in 160 mM NaCl in the pH range of 5.0-6.0 exhibits similar buffer capacity as 6 mM citrate or 14 mM histidine buffers supplied with NaCl to isotonicity. The buffer capacity of antibody solutions scales linearly with protein concentration up to the regime of > 200 mg/ml protein. At 220 mg/ml its buffer capacity resembles the capacity of 30 mM citrate or 50 mM histidine buffer (range of pH 5.0-6.0). The study also provides information on buffer characteristics for pH ranges down to 4.0 and up to 8.0. From theoretical considerations on the buffer capacity of proteins an upper limit of buffering can be inferred. The buffer capacity of monoclonal antibodies is practically identical at the process relevant temperatures 4°C, 25°C and 40°C. Changes in ionic strength of $AI = 0.15$, in contrast, can change the buffer capacity up to 35%. In conclusion, due to efficient self-buffering by antibodies in the pH range of highest chemical stability, conventional buffer excipients are not necessary for high concentrated protein solutions to stabilize the pH of protein solutions. Preferably, the protein/antibody concentration is at least 100 mg/ml or between 100 mg/ml to 220 mg/ml.

**DESCRIPTION OF THE FIGURES**

**FIGURE 1:**
Buffer capacity of the ionizable groups of (A) citrate and (B) histidine (dotted lines, simulated for 50 mM buffering agent) in aqueous solution versus pH and the resulting total buffer capacity (solid line). The total buffer capacity also includes the contribution of the water ionization equilibrium that becomes significant at pH < 3 (dashed line). Due to three carboxyl groups with pKa values that differ around 1 unit, citrate exhibits considerable buffer capacity
over a broad pH range (pH 2.5-6.0). The buffering by the amino, the carboxyl and the imidazole group in histidine hardly overlaps and practically relevant buffer capacity is only found in a narrow range around pH 6.0.

FIGURE 2:
Titration curves of citrate buffer with HCl (A) or NaOH (B) at 25°C and different citrate concentrations. The data values were determined titrating 5 ml of citrate solution with 0.1 or 1 N acid or base. The addition of titrant was normalized to microequivalents of base or acid added per milliliter of solution. Error bars indicate the range for replicates. The lines represent linear fits to the data points. The inverse of the slopes yield the buffer capacity plotted in Figure 3.

FIGURE 3:
The inverse of the slopes from Figure 2 (i.e. the buffer capacities $\beta$, here for the pH-range of 6.0 - 5.0) were plotted versus the corresponding buffer concentration and data points fitted to a linear equation. The slope of capacity (parameter $a$) can be used to compare different buffer systems. In addition, buffer capacities at a certain pH-range can be predicted for various buffer concentrations. Results for histidine, acetate, citrate, succinate and phosphate are summarized in table 3.

FIGURE 4:
Titration curves of mAb3 in 160 mM NaCl at different mAb concentrations. The data values were determined with 5 ml of a given solution using 0.1 or 1 N acid or base at 25°C. The addition of titrant was normalized to microequivalents of base or acid added per milliliter of solution. Error bars indicate the range for replicates. The lines represent linear fits to the data points. The slopes give the buffer capacity plotted in Figure 5.

FIGURE 5:
The experimentally determined buffer capacities ($=\beta$, here for the pH-range of 6.0 - 5.0) of mAb 1-3 and citrate (secondary axis, panel A) or histidine (secondary axis, panel B) were
plotted versus the corresponding concentration. Data points were fitted to a linear equation. The tested antibodies exhibit similar buffering characteristics and their buffer capacity at 50 mg/ml resembles that of 6 mM citrate or 14 mM histidine buffer.

FIGURE 6
Titration curves of mAb4 at different mAb concentrations with NaCl added such that a net osmolality of around 300 mosmol/kg was obtained (i.e. isotonic conditions). The data values were determined with 5 ml of a given solution using 0.1 or 1 N acid or base at 25°C. The addition of titrant was normalized to microequivalents of base or acid added per milliliter of solution. The lines represent linear fits to the data points. The slopes give the buffer capacity plotted in Figure 8.

FIGURE 7
Titration curves of mAb4 at different mAb concentrations with trehalose added such that a net osmolality of around 300 mosmol/kg was obtained (i.e. isotonic conditions). The data values were determined with 5 ml of a given solution using 0.1 or 1 N acid or base at 25°C. The addition of titrant was normalized to microequivalents of base or acid added per milliliter of solution. The lines represent linear fits to the data points. The slopes give the buffer capacity plotted in Figure 8.

FIGURE 8
The experimentally determined buffer capacities (=$\beta$, here for the pH-range of 5.0 - 4.0) of mAb4 in NaCl or trehalose solution (titration curves figures 6 and 7) plotted versus the corresponding mAb concentration. Data points were fitted to a linear equation. The antibody exhibits a linearly increasing buffer capacity up to concentrations above of 200 mg/ml mAb.

FIGURE 9
Titration curves for 10 and 100 mg/ml mAb3 each at 5°C, 25°C and 40°C in the range of pH 4.0-5.0 (A) and pH 5.0-6.0 (B). The addition of titrant was normalized to microequivalents of
acid added per milliliter of solution. The curves and the corresponding slopes (i.e. the inverse of $\beta$) are similar for all temperature conditions.

FIGURE 10

Titrations were performed at 25 °C in the range of pH 5.0-6.0 (A) and 6.0-7.0 (B). The addition of titrant was normalized to microequivalents of acid added per milliliter of solution.

DETAILED DESCRIPTION OF THE INVENTION

Buffering issues, here understood as buffering of pH, are omnipresent throughout the total biopharmaceutical process. Changes in proton concentration can be caused by $\text{CO}_2$ intake, leaching or chemical reactions (Borchert 1989). To nevertheless ensure a stable pH is essential for protecting the therapeutic (e.g. a monoclonal antibody) from degradation during manufacturing, storage and application. Moreover, profound knowledge about buffering characteristics of a system in question is also necessary when controlled pH shifts take place (e.g. in virus inactivation steps, for ion exchange chromatography). This study summarizes buffer characteristics of buffer agents widely used in the biopharmaceutical setting, i.e., acetate, citrate, histidine, succinate, phosphate. Employing the slopes of capacity we summarize here, one can estimate the amount of protons (or hydroxide ions) a buffer system of certain concentration can cope with before the pH is changed and vice versa how many acid/base needs to be added to achieve a requested pH shift. We find that parameters derived via the van Slyke equation (equation 2, vanSlyke 1922) are widely consistent with experimental results. Thus, parameters can actually be calculated without any prior knowledge for these buffering agents employing the equation of van Slyke. At low (and high) pH the ionization equilibrium of water contributes to the total buffer capacity (vanSlyke 1922, cf. Figure 1) but the contribution at the pharmaceutically relevant pH range of pH 4.0-5.0 is negligibly small at buffer concentrations > 10 mM.

The van Slyke equation was derived for the aqueous solution of the buffer agent only. The ionic strength $I$ of the buffer itself and ionization equilibria of additional substances such as NaCl are not considered but will influence the equilibrium of the buffer (cf. simplified Debye
Huckel equation, equation 6, adapted from Scopes 1982). This effect will play a significant role for large changes in ionic strength. As an example the pKa of the histidine imidazole moiety changes from 6.04 at \( I = 0 \) to 6.21 at \( I = 0.5 \). For the range up to isotonicity the effect on buffer capacity close to the pKa seems to be rather negligible. We found an about 10% change in buffer capacity for 25 mM histidine (pH range 5.0-7.0 i.e. around pKa = 6) when changing the NaCl concentration from 10 mM to 140 mM. Equation 6 predicts a change in pKa of 0.06 units under these conditions and the slope of capacity changes by 7% (pH 5.0-6.0) or 5% (pH 6.0-7.0) consistent with the slight change in buffer capacity we have observed in the experiment. According to equation 6 the effect is more pronounced for multiply charged buffers (e.g. for the third ionization of citrate \( z = -2 \)).

\[
p^{K_a} = p^{K_a} + \frac{O.S(2z - 1)\nu^{7}}{1 + LiVF} \]

\( P^{\frac{3}{4}} - pK_a \) value at ionic strength \( I \) with \( z \) being the charge on the acid buffer form

As a matter of fact, uncharged excipients such as commonly used sugars (Hamada 2009), for example 10% α-trehalose in Lucentis® (Genentech, San Francisco USA), do not influence the ionization of the buffer agent via this mechanism.

The upcoming occurrence of high concentrated liquid formulations of monoclonal antibodies suggested the development of self-buffering antibody solutions (Gokarn 2008). In the literature, the characterization of proteins in vitro with respect to buffer attributes widely focuses on determination or prediction of amino acid side chain pKa values and isoelectric points (Tanford 1956, Tanford 1972, McNutt 1990, Baker 1996, Ullmann 1999, Attanasio 1999, Georgescu 2002, Thurlkill 2006b and others). Findings in these studies touch on the subject of buffer capacity indirectly, since any monovalent buffer exhibits a maximum molar buffer capacity of \( \beta = 0.575 \) at the pKa (van Slyke 1922). The buffering power is in principal equal for any (monovalent) buffer, just the pH range in which a certain average buffer capacity is observed, is specific. In addition, comprehensive information on buffer attributes can be found for proteins in physiological settings (vanSlyke 1922, Hainsworth 1986, Leem 1999, Lamanda 2007 and others). Findings on buffer capacity of antibodies in vitro for pH ranges down to pH 4.0 and up to 8.0 are summarized in the current study. The prediction of buffering power by antibodies is not straightforward. Consistently with results of others
(Gokarn 2008), models employed here that sum up the contributions of histidine; aspartate and glutamate moieties in the protein overestimate the buffer capacity. Thus, the theoretically derived values might serve as setting an upper limit. In context of the protein tertiary structure pKa values of the amino acid side chains can change drastically due to charged neighbours, hydrogen bonding or dehydration effects (Kuramitsu 1980, McNutt 1990, Forsyth 2002, Thurlkill 2006b). Changes of up to 3 units have been observed for proteins (Asp76 in RNase T1 pKa = 0.5 Pace 1998). Similar phenomena are likely to occur in the studied antibodies and would totally diminish the buffering contribution of certain amino acids in the biopharmaceutically favored pH range of pH 4.0-7.0. This might explain why the apparent buffer capacity of the proteins is lower than inferred theoretically employing pKa values that were determined from model compounds.

The studied antibodies exhibit strong buffering in the range of pH 4.0-6.0. This range is within the pH range of maximal chemical stability of proteins (pH 4.0-7.0) and is advisable for a self-buffering formulation. The buffer capacities we determined experimentally indicate the antibodies can sufficiently buffer proton changes due to leaching occurring during storage and handling (Preston 1984, Borchert 1989, White 2008). An antibody solution of 50 mg/ml at pH 6 buffers around 1 mM of protons before the pH drops to pH 5.75. This amount surpasses the amount of protons leached from borosilicate glass vials by a factor of 100 (Bahrenburg & Garidel unpublished observations).

Biopharmaceuticals are handled at room temperature, stored at 5°C and kept at up to 40°C in stability studies thus the effect of temperature on buffer capacity needs to be considered. The temperature dependence of an equilibrium reaction is factorized via the enthalpy of the ionization reaction. If the standard molar ionization enthalpy is close to zero (e.g. for carboxyl groups and phosphoric acids as well as organic derivatives of them Goldberg 2002) pKa values are hardly affected by temperature changes. The buffer capacity in the tested antibodies is for the most part provided by the carboxyl side chains of aspartate and glutamate (table 2). Consistently, we found that temperature changes in the range of 5 to 40°C do not affect the buffer capacity of monoclonal antibodies in the range of pH 4.0-8.0.

In contrast, the influence of ionic strength on self-buffering proteins can be considerable. For lysozyme considerable changes on the titration curve were observed in the range of pH 1.5-
4.5 for ionic strengths of $7 = 1$ and $I = 0.1$. Effects were only minimal between pH 4.5 and 9 (Tanford & Roxby 1972). Moderate changes on the titration curve were also observed for Ribonuclease at ionic strength of $7 = 0.01$ to $7 = 0.15$ (Tanford 1956) and cytochrome c at 0 M, 0.1 M and 0.5 M NaCl (Bostrom 2006). We found deviating titration curves (pH 5.0-7.0) and therefore different buffer capacities in that pH range for a monoclonal antibody when changing NaCl concentration from 160 mM to 10 mM ($7 = 0.16$ and 0.01 respectively neglecting the contribution of charged antibody to the total ionic strength of the solution). Such effects need to be considered for example during purification processes. At storage and handling practically no changes in ionic strength occur. The use of self-buffering high concentrated antibody solution might thus be first of all recommended for the end formulation or as a preliminary preparation before end formulation (Mahler 2010). In stability studies of Gokarn and colleagues the pH of a self-buffering antibody solution remained constant over a 12 month storage, formation of soluble aggregates at 50 °C was least for the self-buffered in comparison to the conventionally buffered solutions and the antibody was not compromised by freeze-thaw cycles (Gokarn 2008). In this study, sorbitol was added to the antibody solution to ensure isotonicity. In the present study isotonicity conditions were approximated by including NaCl or trehalose in the solution. Transferring the protein into excipient free solutions may induce phase separation upon exchange into pure water (Trilisky 2011). Leaving out buffer agents in high concentrated protein formulations appears to be advantageous for several reasons. When employing self-buffering formulations the toxicological evaluation of buffer excipients is not necessary thus saving time and costs. Discoloration events by buffer components (Chen 2003) and chemical instability reactions caused by impurities in buffer excipients can be avoided. Moreover, the physico-chemical stability of the antibody might be even improved as an increasing concentration of conventional buffer has proved to sometimes enhance aggregation (Gokarn 2008). However, destabilizing as well as stabilizing effects have been attributed to buffer components (Manning 2010). Hence, case to case assessments are still advisable.
The inverse value of the slope yields the average buffer capacity $\beta$ for the chosen pH range. The buffer capacity describes how many microequivalents can be added to 1 ml of the buffer or antibody system before changing the pH one unit. For theoretical determination of the buffer capacity the equation of van Slyke was used. According to van Slyke the buffer capacity $C$ of an aqueous solution is described by equations (1) and (2) (vanSlyke 922).

$$\beta = 2.3 \left( \frac{K_{a1} [H^+]}{K_{a2} + [H^+] + [OH^-]} + [H^+] + [OH^-] \right)$$

$$\beta = 2.3C[H^+ \left( \frac{K_{a1}}{K_{a2} + [H^+]} + \frac{K_{a2}}{K_{a3} + [H^+]^2} + \ldots \right) + 2.3 \left( [H^+] + [OH^-] \right)]$$

Here, $C$ is the total buffer concentration and $K_a$ is the acid constant of the buffer. Equation 1 is employed to buffer systems that are characterized by a single pKa value only. More complex systems are described by equation 2 adding up various pKa values to a total buffer capacity. As an example, the effect of three ionizable groups in citrate and histidine on the total buffer capacity is depicted in Figure 1A and 1B respectively. In citrate the pKa values of three carboxyl groups are around 1 unit apart leading to a constantly large buffer capacity over a broad range (pH 2.5-6.0) (Figure 1A). In contrast, the pKa values of the amino group, the carboxyl group and the imidazole moiety in histidine differ up to 4 units. Thus, the buffer effects of these groups hardly overlap and significant buffer capacity is found in narrow pH ranges only (Figure 1B).

Equation 2 yields the buffer capacity for discrete pH values. Therefore, the calculated average value within $\Delta$pH=1 or 0.5 (as indicated) was compared with the experimentally determined value for the same range.

For calculation of the buffering capacity of an antibody equation 3 has been suggested by Gokarn and colleagues (Gokarn2008).

$$\beta_{mAb} = \sum_{i=A}^{N_i=Asp} \left( \frac{[H^+]_{i\ pH}}{[K_{a1} + [H^+]]_{i\ pH}} - \frac{[H^+]_{i\ pH+1}}{[K_{a2} + [H^+]]_{i\ pH+1}} \right) + \left( [H^+]_{pH} - [H^+]_{pH+1} \right)$$

In equation 3, the subscript i refers to the amino acids aspartic acid, glutamic acid and histidine (relevant for the buffering within the pH range of interest), $N$ is the number of amino acids of the mAb, $C_{mAb}$ is the molar concentration of mAb and $K_a$ is the acid dissociation.
constant of the corresponding amino acid side-chain. The indices "pH" and "pH+1" denote the start and end value of the specific pH range $\Delta pH = 1$ being addressed.

In Figure 5 the experimentally determined buffer capacities are plotted versus the concentration of mAb1, mAb2 and mAb3. Since the buffer capacity increases linearly with concentration, just as found for conventional buffers, the data points were fitted to a linear equation. The slope i.e. the slope of capacity was determined and turns out to be nearly identical for mAb1, mAb2 and mAb3. This similarity was found for the overall pH-range of 4.0-8.0 (cf. Table 4).

The graphs in Figure 5A and 5B also include the experimentally determined buffer capacities of the conventional buffer citrate and histidine respectively at different concentrations in the pH-range pH 6.0-5.0 (secondary axis). We find that the monoclonal antibodies exhibit inherent buffer capacity that at 50 mg/ml resembles the capacity of 6 mM citrate or 14 mM histidine buffer in the pH-range of pH 6.0-5.0. As 10 mM histidine is a commonly used buffer (e.g. end-formulation of Rituximab (Wang2007)), we conclude the self-buffering effect of a 50 mg/ml antibody solution is sufficient to replace the buffer excipient.

Table 5 summarizes the corresponding values from titrations of antibody solutions containing NaCl or trehalose. We find a linear increase of buffer capacity with increasing protein concentration up to more than 200 mg/ml under conditions close to isotonicity. In the pH range of pH 4.0-5.0 the antibody solution of 220 mg/ml protein in trehalose or NaCl exhibits a buffer capacity resembling the capacity of around 60 mM citrate buffer. In the range of pH 5.0-6.0 this highly concentrated antibody solution buffers like 50 mM histidine or 30 mM citrate buffer. These values underline that the buffer capacity of the protein can substitute for a buffering excipient in highly concentrated antibody solutions. Hence, self-buffering antibody solutions comprising a therapeutic antibody at a concentration of at least 100 mg/ml are capable of maintaining a stable pH for the purpose of stabilizing the antibody solution during long-term storage.
The buffer capacity is nearly identical for all temperatures. This holds true for titrations at protein concentrations of 10 mg/ml and 100 mg/ml. Also, in the range of pH 6.0-7.0 and pH 7.0-8.0 a nearly identical buffer capacity was determined from titrations at 5 °C, room temperature and 40 °C (data not shown). The experiments indicate the inherent buffer capacity of mAbs is not significantly altered upon temperature changes within the range relevant for biopharmaceutic processes.

According to the Debye-Hückel theory (Debyel923) the activity coefficients of the species in an ionization equilibrium and therefore the pKa of acids depend on the ionic strength of a solution. A change in ionic strength can thus alter the average buffer capacity of a defined pH range. To check for the practical relevance of this effect, we determined average buffer capacities of buffers for different ionic strength conditions. We find that a change in ionic strength in the range of 10 mM NaCl to 160 mM NaCl (commonly employed in mAb formulations) has a moderate effect on the buffer capacity of mAb3 in the range of pH 5.0-6.0 and pH 6.0-7.0 (e.g. see example 4: change in β around 35 % and 25 % respectively).

This is especially relevant for a dilution application, such as the preparation of a solution for administration to a patient (e.g. by infusion), wherein an aqueous solution is used for infusion into a subject / patient, which is based on a) a concentrate of the therapeutic antibody, i.e. a small volume of 0.1 ml to 20 ml, or 1 ml to 10 ml, preferably 1 ml of a composition provided herein (> 100 mg/ml protein concentration), and b) a larger volume of e.g. 250 ml, 500 ml or between 50 ml to 1 L, 100 ml to 500 ml, 250 ml to 500 ml of a solution. This larger volume of a solution is preferably an isotonic solution.

DEFINITIONS

Terms not specifically defined herein should be given the meanings that would be given to them by one of skill in the art in light of the disclosure and the context. The general embodiments "comprising" or "comprised" encompass the more specific embodiment "consisting of. Furthermore, singular and plural forms are not used in a limiting way. As used in the specification, however, unless specified to the contrary, the following terms have the meaning indicated and the following conventions are adhered to.
Terms used in the course of this present invention have the following meaning.

The term "protein" is used interchangeably with amino acid residue sequences or polypeptide and refers to polymers of amino acids of any length. These terms also include proteins that are post-translationally modified through reactions that include, but are not limited to, glycosylation, acetylation, phosphorylation or protein processing. Modifications and changes, for example fusions to other proteins, amino acid sequence substitutions, deletions or insertions, can be made in the structure of a polypeptide while the molecule maintains its biological functional activity. For example certain amino acid sequence substitutions can be made in a polypeptide or its underlying nucleic acid coding sequence and a protein can be obtained with like properties.

The term "antibody" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant regions genes as well as the myriad immunoglobulin variable region genes.

As used herein, the term "antibody" includes a polyclonal, monoclonal, bi-specific, multi-specific, human, humanized, or chimeric antibody. The terms "antibody" and "immunoglobulin" are used interchangeably.

The term "antibody" is used in the broadest sense. The term "antibody" comprises full-length immunoglobulin molecules as well as immunoglobulin based molecules such as immunoglobulin fragments, immunoglobulin isoforms, fusion proteins of immunoglobulin chains, immunoglobulins conjugated to a non-proteinaceous moiety. The term "antibody" specifically covers single monoclonal antibodies (including agonist and antagonist antibodies) and antibody compositions with polyepitopic specificity. The term "antibody" specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies) and antibody fragments. Exemplary antibodies within the scope of the present invention include but are not limited to
anti-CD20, anti-CD33, anti-CD37, anti-CD40, anti-CD44, anti-CD52, anti-HER2/neu (erbB2), anti-EGFR, anti-IGF, anti-VEGF, anti-TNFalpha, anti-IL2 or anti-IgE antibodies. The term "monoclonal antibody" (mAb) as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies based on the amino acid sequence. Monoclonal antibodies are highly specific, being direct against a single antigenic site.

Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each mAb is directed against a single determinant on the antigen. In addition to their specificity, the mAbs are advantageous in that they can be synthesized by cell culture (hybridomas, recombinant cells or the like) uncontaminated by other immunoglobulins. The mAbs herein include for example chimeric, humanized and human antibodies.

"Chimeric antibodies" are antibodies whose light and/or heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin variable and constant regions belonging to identical or homologous to corresponding sequences of different species, such as mouse and human. Or alternatively, whose heavy chain genes are belonging to a particular antibody class or subclass while the remainder of the chain is from another antibody class or subclass of the same or from another species. It covers also fragments of such antibodies. For example, the variable segments of the genes from a mouse monoclonal antibody may be joined to human constant segments, such as gamma 1 and gamma 3. A typical therapeutic chimeric antibody is thus a hybrid protein composed of the variable or antigen-binding domain from a mouse antibody and the constant or effector domain from a human antibody (e.g. ATCC Accession No. CRL 9688 secretes an anti-Tac chimeric antibody), although other mammalian species may be used.

The term "humanized antibodies" according to the present invention refers to specific chimeric antibodies, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab’, F(ab)2 or other antigen-binding subsequences of antibodies), and which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary -
determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by the corresponding non-human residues. Furthermore, humanized antibodies can comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin.

Humanized antibody: comprising a human framework region and one or more CDRs from a non-human (usually a mouse or rat) antibody. Adjustments in framework amino acids might be required to keep antigen binding specificity, affinity and or structure of domain.

Reference is made to the domain structure of immunoglobulins as applied to human IgG1 by Kabat, E.A. (Kabat, 1988; Kabat et al., 1991). Full length "immunoglobulins" are generally heterotetrameric glycoproteins of about 150 kDa, composed of two identical light and two identical heavy chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of different immunoglobulins isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has an amino terminal variable domain (VH) followed by carboxy terminal constant domains (CH). Each light chain has a variable N-terminal domain (VL) and a C-terminal constant domain (CL).

Depending on the amino acid sequence of the constant domain of the heavy chains, antibodies can be assigned to different classes. There are five major classes: IgA, IgD, IgE, IgG and IgM. The heavy chain constant domains that correspond to the different classes of antibodies are called alpha, delta, epsilon, gamma and mu domains, respectively. The mu chain of IgM contains five domains (VH, CHmul, CHmu2, CHmu3 and CHmu4). The heavy chain of IgE
also contains five domains while the heavy chain of IgA has four domains. The
immunoglobulin class can be further divided into subclasses (isotypes), e.g. IgGl, IgG2,
IgG3, IgG4, IgAl and IgA2.
The Fc region of a full antibody usually comprises two CH2 domains and two CH3 domains.
The amino acid sequences of immunoglobulin CH2 domains are known or are generally
available to the skilled artisan (Kabat et al., 1991). A preferred immunoglobulin CH2 domain
within the context of the present invention is a human IgG and preferably from IgGl, IgG2,
IgG3, IgG4, more preferably a human IgGl and IgG3 and even more preferred a human IgGl.
Using the numbering system of Edelman (Edelman et al., 1969), the immunoglobulin CH2
domain preferably begins at amino acid position equivalent to glutamine 233 of human IgGl
and extends through amino acid equivalent to lysine 340 (Ellison and Hood, 1982).
With respect to human antibody molecules reference is made to the IgG class in which an N-
linked oligosaccharide is attached to the amide side chain of Asn 297 of the beta-4 bend to the
inner face of the CH2 domain of the Fc region. It is characteristic of the glycoprotein,
especially the antibody or Fc-fusion protein of the present invention that it contains or be
modified to contain at least a CH2 domain. The CH2 domain is a CH2 domain of an
immunoglobulin having a single N-linked oligosaccharide of a human IgG CH2 domain. The
CH2 domain is preferably the CH2 domain of human IgGl.
Especially, desired proteins/polypeptides are for example, but not limited to insulin, insulin-
like growth factor, hGH, tPA, cytokines, such as interleukines (IL), e.g. IL-1, IL-2, IL-3, IL-4,
IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18,
interferon (IFN) alpha, IFN beta, IFN gamma, IFN omega or IFN tau, tumor necrosis factor
(TNF), such as TNF alpha and TNF beta, TNF gamma, TRAIL; G-CSF, GM-CSF, M-CSF,
MCP-1, VEGF, and single domain antibodies (camelid derived antibodies). Also included is
the production of erythropoietin or any other hormone growth factors and any other
polypeptides that can serve as agonists or antagonists and/or have therapeutic or diagnostic
use.

"Fc-fusion proteins" are defined as proteins which contain or are modified to contain at least
the portion of the CH2 domain of the heavy chain immunoglobulin constant region
comprising the single N-linked glycosylation site. According to the Kabat EU nomenclature (Kabat et al, 1991) this position is Asn297 in an IgGl, IgG2, IgG3 or IgG4 antibody. The other part of the fusion protein can be the complete sequence or any part of the sequence of a natural or modified heterologous protein or a composition of complete sequences or any part of the sequence of natural or modified heterologous protein proteins. The immunoglobulin constant domain sequences may be obtained from any immunoglobulin subtypes, such as IgGl, IgG2, IgG3, IgG4, IgAl or IgA2 subtypes or classes such as IgA, IgE, IgD or IgM. Preferentially they are derived from human immunoglobulin, more preferred from human IgG and even more preferred from human IgGl and IgG3. Examples of Fc fusion proteins comprise MCPI-Fc, ICAM-Fc, EPO-Fc, scFv fragments or the like coupled to the CH2 domain of the heavy chain immunoglobulin constant region comprising the N-linked glycosylation site. Fc-fusion proteins can be constructed by genetic engineering approaches by introducing the CH2 domain of the heavy chain immunoglobulin constant region comprising the N-linked glycosylation site into another expression construct comprising for example other immunoglobulin domains, enzymatically active protein portions, or effector domains. Thus, an Fc fusion protein according to the present invention comprises also a single chain Fv (scFv) fragment linked to the CH2 domain of the heavy chain immunoglobulin constant region comprising e.g. the N-linked glycosylation site.

Furthermore, antibody fragments include e.g. "Fab fragments" (Fragment antigen-binding = Fab). Fab fragments consist of the variable regions of both chains which are held together by the adjacent constant region. These may be formed by protease digestion, e.g. with papain, from conventional antibodies, but similar Fab fragments may also be produced in the mean time by genetic engineering. Further antibody fragments include F(ab')2 fragments, which may be prepared by proteolytic cleaving with pepsin. Antibody fragments also include Fc fragments, and scFv fragments, as well as other antibody derivatives described further herein below.

Using genetic engineering methods it is possible to produce shortened antibody fragments which consist only of the variable regions of the heavy (VH) and of the light chain (VL).
These are referred to as Fv fragments (Fragment variable = fragment of the variable part). Since these Fv-fragments lack the covalent bonding of the two chains by the cysteines of the constant chains, the Fv fragments are often stabilised. It is advantageous to link the variable regions of the heavy and of the light chain by a short peptide fragment, e.g. of 10 to 30 amino acids, preferably 15 amino acids. In this way a single peptide strand is obtained consisting of VH and VL, linked by a peptide linker. An antibody protein of this kind is known as a single-chain-Fv (scFv).

In recent years, various strategies have been developed for preparing scFv as a multimeric derivative. This is intended to lead, in particular, to recombinant antibodies with improved pharmacokinetic and biodistribution properties as well as with increased binding avidity. In order to achieve multimerisation of the scFv, scFv were prepared as fusion proteins with multimerisation domains. The multimerisation domains may be, e.g. the CH3 region of an IgG or coiled coil structure (helix structures) such as Leucin-zipper domains. However, there are also strategies in which the interaction between the VH/VL regions of the scFv are used for the multimerisation (e.g. dia-, tri- and pentabodies). By diabody the skilled person means a bivalent homodimeric scFv derivative. The shortening of the Linker in a scFv molecule to 5-10 amino acids leads to the formation of homodimers in which an inter-chain VH/VL-superimposition takes place. Diabodies may additionally be stabilised by the incorporation of disulphide bridges.

By minibody the skilled person means a bivalent, homodimeric scFv derivative. It consists of a fusion protein which contains the CH3 region of an immunoglobulin, preferably IgG, most preferably IgGl as the dimerisation region which is connected to the scFv via a Hinge region (e.g. also from IgGl) and a Linker region.

By triabody the skilled person means a trivalent homotrimERIC scFv derivative. ScFv derivatives wherein VH-VL are fused directly without a linker sequence lead to the formation of trimers.

The skilled person will also be familiar with so-called miniantibodies which have a bi-, tri- or tetravalent structure and are derived from scFv. The multimerisation is carried out by di-, tri- or tetrameric coiled coil structures.
By "scaffold proteins" a skilled person means any functional domain of a protein that is coupled by genetic cloning or by co-translational processes with another protein or part of a protein that has another function.

The term "water" means liquid hydrogen oxide in its meaning in the chemical arts. WFI (=water for injection) is defined as water purified as described in the European Pharmacopoeia 7.5.

The term "composition" means a mixture of substances.

The term "pharmaceutical composition" means a mixture of substances including the therapeutically active substance for pharmaceutical use.

The compositions/pharmaceutical compositions of the present invention may be used to treat cancer or other abnormal proliferative diseases. Cancers are classified in two ways: by the type of tissue in which the cancer originates (histological type) and by primary site, or the location in the body where the cancer first developed. The most common sites in which cancer develops include the skin, lungs, female breasts, prostate, colon and rectum, the lymphoid system, cervix and uterus.

The compositions are thus useful in the treatment of a variety of cancers, including but not limited to the following: AIDS-related cancer such as Kaposi's sarcoma; bone related cancer such as Ewing's family of tumors and osteosarcoma; brain related cancer such as adult brain tumor, childhood brain stem glioma, childhood cerebellar astrocytoma, childhood cerebral astrocytoma/malignant glioma, childhood ependyymoma, childhood medulloblastoma, childhood supratentorial primitive neuroectodermal tumors, childhood visual pathway and hypothalamic glioma and other childhood brain tumors; breast cancer; digestive/gastrointestinal related cancer such as anal cancer, extrahepatic bile duct cancer, gastrointestinal carcinoid tumor, colon cancer, esophageal cancer, gallbladder cancer, adult primary liver cancer, childhood liver cancer, pancreatic cancer, rectal cancer, small intestine cancer and stomach (gastric) cancer; endocrine related cancer such as adrenocortical carcinoma, gastrointestinal carcinoid tumor, islet cell carcinoma (endocrine pancreas),
parathyroid cancer, pheochromocytoma, pituitary tumor and thyroid cancer; eye related
cancer such as intraocular melanoma, and retinoblastoma; genitourinary related cancer such
as bladder cancer, kidney (renal cell) cancer, penile cancer, prostate cancer, transitional cell
renal pelvis and ureter cancer, testicular cancer, urethral cancer, Wilms' tumor and other
childhood kidney tumors; germ cell related cancer such as childhood extracranial germ cell
tumor, extragonadal germ cell tumor, ovarian germ cell tumor and testicular cancer;
gynecologic related cancer such as cervical cancer, endometrial cancer, gestational
 trophoblastic tumor, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low
malignant potential tumor, uterine sarcoma, vaginal cancer and vulvar cancer; head and neck
related cancer such as hypopharyngeal cancer, laryngeal cancer, lip and oral cavity cancer,
metastatic squamous neck cancer with occult primary, nasopharyngeal cancer, oropharyngeal
cancer, paranasal sinus and nasal cavity cancer, parathyroid cancer and salivary gland cancer;
hematologic/blood related cancer such as leukemias, such as adult acute lymphoblastic
leukemia, childhood acute lymphoblastic leukemia, adult acute myeloid leukemia, childhood
acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia and
hairy cell leukemia; and lymphomas, such as AIDS-related lymphoma, cutaneous T-cell
lymphoma, adult Hodgkin's lymphoma, childhood Hodgkin's lymphoma, Hodgkin's
lymphoma during pregnancy, mycosis fungoides, adult non-Hodgkin's lymphoma, childhood
non-Hodgkin's lymphoma, non-Hodgkin's lymphoma during pregnancy, primary central
nervous system lymphoma, Sezary syndrome, cutaneous T-cell lymphoma and Waldenstrom's
macroglobulinemia and other hematologic/blood related cancer such as chronic
myeloproliferative disorders, multiple myeloma/plasma cell neoplasm, myelodysplastic
syndromes and myelodysplastic/myeloproliferative diseases; lung related cancer such as non-
small cell lung cancer and small cell lung cancer musculoskeletal related cancer such as
Ewing's family of tumors, osteosarcoma, malignant fibrous histiocytoma of bone, childhood
rhabdomyosarcoma, adult soft tissue sarcoma, childhood soft tissue sarcoma and uterine
sarcoma; neurologic related cancer such as adult brain tumor, childhood brain tumor, brain
stem glioma, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma,
medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and
hypothalamic glioma and other brain tumors such as neuroblastoma, pituitary tumor and
primary central nervous system lymphoma; respiratory/thoracic related cancer such as non-small cell lung cancer, small cell lung cancer, malignant mesothelioma, thymoma and thymic carcinoma; skin related cancer such as cutaneous T-cell lymphoma, Kaposi's sarcoma, melanoma, Merkel cell carcinoma and skin cancer.

These disorders have been well characterized in man, but also exist with a similar etiology in other mammals, and can be treated by pharmaceutical compositions of the present invention.

For therapeutic use, the compositions/pharmaceutical compositions of the present invention may be administered in a therapeutically effective amount in any conventional dosage form in any conventional manner. The following parenteral routes of administration are useful in various embodiments of the invention: administration by intravenous, intraarterial, intracardiac, intraspinal, intrathecal, intraosseous, intraarticular, intrasynovial, intracutaneous, intradermal, subcutaneous, peritoneal, and/or intramuscular injection.

A therapeutically effective amount can be determined by a skilled artisan based upon such factors as weight, metabolism, and severity of the affliction etc. Preferably the active compound is dosed at about 1 mg to about 500 mg per kilogram of body weight on a daily basis. More preferably the active compound is dosed at about 1 mg to about 100 mg per kilogram of body weight on a daily basis.

FURTHER EMBODIMENTS

The invention provides a method of administering a composition to a subject comprising the following steps:

a. Providing a composition comprising a protein, preferably an antibody or antibody fragment or Fc-fusion protein, at a concentration of at least 100 mg/ml, wherein said composition does not contain a buffer excipient, having a volume of 0.1 ml to 20 ml, or 1 ml to 10 ml, preferably 1 ml,

b. Diluting said composition of step a) into a larger volume of a solution, wherein the larger volume is between 50 ml to 1 L, or between 100 ml to 500 ml, preferably 250 ml, or 500 ml, or 250 ml to 500 ml,

c. Administering said solution of step b) to a subject.
The invention also provides a method of preparing a composition for administration to a
subject comprising the following steps:

a. Providing a composition comprising a protein, preferably an antibody or antibody
fragment or Fc-fusion protein, at a concentration of at least 100 mg/ml, wherein said
composition does not contain a buffer excipient, having a volume of 0.1 ml to 20 ml,
or 1 ml to 10 ml, preferably 1 ml,
b. Diluting said composition of step a) into a larger volume of a solution, wherein the
larger volume is between 50 ml to 1 L, or between 100 ml to 500 ml, preferably 250
ml, or 500 ml, or 250 ml to 500 ml.

The invention concerns a (pharmaceutical) composition comprising a (pharmaceutical and/or
therapeutical) protein, preferably an antibody or antibody fragment or Fc-fusion protein, at a
concentration of at least 100 mg/ml, wherein said composition does not contain a buffer
excipient. Preferably said protein is a pharmaceutical protein. Furthermore, preferably said
protein is a therapeutic protein.

The invention concerns a (pharmaceutical) composition comprising a (pharmaceutical)
protein, preferably an antibody or antibody fragment or Fc-fusion protein, at a concentration
of at least 100 mg/ml, wherein the buffer concentration in the composition is 0 mg/ml.

The invention concerns a (pharmaceutical) composition comprising:

a) a (pharmaceutical) protein, preferably an antibody or antibody fragment or Fc-
fusion protein, at a concentration of at least 100 mg/ml and
b) water, preferably WFI water,
c) but no buffer salts.

In one embodiment of the methods according to the invention, a (pharmaceutical)
composition comprising:

a) a (pharmaceutical) protein, preferably an antibody or antibody fragment or Fc-
fusion protein, at a concentration of at least 100 mg/ml and
b) water, preferably WFI water,
c) but no buffer salts is used.

In a specific embodiment of the compositions and methods of the invention said composition
is an isotonic solution for example comprising salt like NaCl and/or sugar components like
trehalose or saccharose. Preferably, said composition is an isotonic solution which does not contain buffer excipients. In a specific embodiment said isotonic solution has a volume of 0.1 ml to 20 ml, or 1 ml to 10 ml, preferably 1 ml.

In a specific embodiment of the compositions and methods of the invention said composition has an osmolarity of approximately 308 mosmol/l / an osmolality of around 300 mosmol/kg. Preferably said composition is a liquid. Specifically said composition is a liquid formulation. More specifically said composition is an aqueous solution. Preferably, said aqueous solution is an isotonic solution such as 0.9% m/V NaCl in water. Preferably said liquid formulation/aqueous solution buffers changes in pH in the absence of a buffer excipient.

In a specific embodiment of the compositions and methods of the invention the protein/antibody provides 100% of the buffer capacity of said composition.

In another embodiment of the compositions and methods of the present invention the composition is non-isotonic. Preferably, said non-isotonic composition is a non-isotonic solution. In a specific embodiment said non-isotonic solution has a volume of 0.1 ml to 20 ml, or 1 ml to 10 ml, preferably 1 ml.

In a further embodiment of the compositions and methods according to the invention the only other component of the composition besides the protein component is water (H₂O), preferably water for injection (WFI). Preferably said composition comprising only protein (e.g. antibody) and water has a volume of 0.1 ml to 20 ml, or 1 ml to 10 ml, preferably 1 ml.

In a specific embodiment of the present invention said non-isotonic composition and/or said composition comprising only protein and water is diluted into an excess volume / a larger volume of a buffer solution and/or an isotonic solution. In a specific embodiment of the present invention said non-isotonic composition and/or said composition comprising only protein and water is diluted into an excess volume / a larger volume of an isotonic solution which does not contain a buffer excipient. Preferably, the composition of the invention is diluted into an excess volume / a larger volume of an isotonic solution, preferably with an osmolarity of around 308 mosmol/l / an osmolality of around 300 mosmol/kg, which does not contain a buffer excipient. Preferably said isotonic solution is a physiological salt solution (9 g NaCl in 1 Liter (0.9 % m/V) of water, preferably with an osmolarity of around 308 mosmol/l / an osmolality of around 300 mosmol/kg). In a specific embodiment of the
present invention said excess volume / larger volume of a buffer solution and/or an isotonic solution has a volume of 250 ml, 500 ml or between 50 ml to 1 L, 100 ml to 500 ml, or 250 ml to 500 ml. In a specific embodiment of the present invention said excess volume / larger volume of a solution does not contain a buffer excipient.

In a specific embodiment the protein is a therapeutic protein, preferably a therapeutic antibody, an antibody fragment, an Fc-fusion protein, a full-length immunoglobulin molecule, an immunoglobulin based molecule such as an immunoglobulin fragment, an immunoglobulin isoform, a fusion protein comprising at least one immunoglobulin chain, or an immunoglobulin conjugated to a non-proteinaceous moiety.

In a specific embodiment the protein (preferably an antibody) concentration is at least 110 mg/ml, at least 115 mg/ml, at least 120 mg/ml, at least 150 mg/ml, at least 200 mg/ml, at least 220 mg/ml, at least 300 mg/ml or within the range of 100 mg/ml to 220 mg/ml or 100 mg/ml to 300 mg/ml.

In a further embodiment of the present invention at the pH of the composition, 25°C, one atmosphere, and equilibrium with ambient atmosphere, the protein has a buffer capacity of at least that of approximately 4.0 mM citrate buffer in the pH range of pH 4.0 to 7.0 under the same conditions. Preferably the protein concentration is approximately 100 mg/ml. Preferably the composition has been approved for pharmaceutical use by an authority legally empowered to grant such approval.

In a further embodiment of the present invention at the pH of the composition, 25°C, one atmosphere, and equilibrium with ambient atmosphere, the protein has a buffer capacity of approximately 60 mM citrate buffer in the pH range of pH 4.0 to 5.0 under the same conditions. Preferably the protein concentration is approximately 220 mg/ml. Preferably the composition has been approved for pharmaceutical use by an authority legally empowered to grant such approval.

In further embodiments of the present invention (at the pH of the composition, 25°C, one atmosphere, and equilibrium with ambient atmosphere) the protein/antibody has a buffer capacity as listed below:
<table>
<thead>
<tr>
<th>pH-range</th>
<th>cone. protein/mAb [mg/ml]</th>
<th>cone. Citrate [mM]</th>
<th>buffer capacity [µEq/µmol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>26</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>40</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>53</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>66</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>79</td>
<td>62</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH-range</th>
<th>cone. protein/mAb [mg/ml]</th>
<th>cone. Histidine [mM]</th>
<th>buffer capacity [µEq/µmol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>14</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>21</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>28</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>35</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>42</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH-range</th>
<th>cone. protein/mAb [mg/ml]</th>
<th>cone. Phosphate [mM]</th>
<th>buffer capacity [µEq/µmol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>15</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>20</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>25</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>30</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH-range</th>
<th>cone. protein/mAb [mg/ml]</th>
<th>cone. Phosphate [mM]</th>
<th>buffer capacity [µEq/µmol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>12</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>17</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>23</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>29</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>35</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

In the pH range of pH 4.0-5.0 the antibody solution of 220 mg/ml protein in trehalose or NaCl exhibits a buffer capacity resembling the capacity of around 60 mM citrate buffer. In the pH
range of pH 5.0-6.0 the antibody solution of 220 mg/ml protein in trehalose or NaCl exhibits a buffer capacity resembling the capacity of around 50 mM histidine buffer or around 30 mM citrate buffer.

In a further embodiment of the present invention the pH of the composition maintained by the buffering action of the protein is between approximately pH 4.0 and pH 8.0 (see table 4), preferably pH 4.0 to pH 7.0, or pH 4.0 to pH 6.0, or pH 5.0 to 7.0. More preferably, the pH of the composition maintained by the buffering action of the protein is pH 4.0 to pH 5.0, pH 5.0 to pH 6.0, or pH 6.0 to pH 7.0. In specific embodiments the pH of the solution obtained in step b) is between approximately pH 4.0 and pH 8.0, preferably pH 4.0 to pH 7.0, or pH 4.0 to pH 6.0, or pH 5.0 to 7.0. More preferably, the pH of the solution obtained in step b) is pH 4.0 to pH 5.0, pH 5.0 to pH 6.0, or pH 6.0 to pH 7.0.

In a further embodiment of the present invention the antibody is a full-length immunoglobulin molecule, an immunoglobulin based molecule such as an immunoglobulin fragment, an immunoglobulin isoform, a fusion protein comprising at least one immunoglobulin chain, or an immunoglobulin conjugated to a non-proteinaceous moiety.

In a further embodiment of the methods according to the invention the composition comprising a protein at a concentration of at least 100 mg/ml is provided by reconstitution of a lyophilisate and/or by removing residual buffer excipients, preferably via dialysis or ultrafiltration/diafiltration (UF/DF).

In a further aspect, one or more further proteins may be added as a separate composition prior to or subsequently to diluting said composition of step a) of the methods of the invention into a larger volume of a solution, or as part of the larger volume of a solution used for the dilution of the composition of step a). Said one or more further proteins may be selected from the group consisting of a therapeutic protein, preferably a therapeutic antibody, an antibody fragment, an Fc-fusion protein, a full-length immunoglobulin molecule, an immunoglobulin based molecule such as an immunoglobulin fragment, an immunoglobulin isoform, a fusion protein comprising at least one immunoglobulin chain, or an immunoglobulin conjugated to a non-proteinaceous moiety. Preferably, the one or more further proteins are added as a separate
composition comprising the one or more further proteins at a (total) concentration of at least 100 mg/ml. In certain embodiments, the one or more further proteins may provide 100% of the buffer capacity of the separate composition comprising the one or more further proteins. In specific embodiments of the invention, the protein, or the protein and the one or more further proteins, provide/s 100% of the buffer capacity of the solution obtained in step b) of the methods according to the invention.

According to the invention, the (total) concentration of the one or more further proteins in the separate composition may be at least 110 mg/ml, at least 115 mg/ml, at least 120 mg/ml, at least 150 mg/ml, at least 200 mg/ml, at least 220 mg/ml, at least 300 mg/ml or within the range of 100 mg/ml to 220 mg/ml or 100 mg/ml to 300 mg/ml.

The invention furthermore provides a kit comprising in one or more containers a composition according to the invention comprising a protein, preferably an antibody or antibody fragment or Fc-fusion protein, at a concentration of at least 100 mg/ml, wherein said composition does not contain a buffer excipient, having a volume of 0.1 ml to 20 ml, or 1 ml to 10 ml, preferably 1 ml, and a larger volume of a solution according to the invention wherein the larger volume is between 50 ml to 1 L, or between 100 ml to 500 ml, preferably 250 ml, or 500 ml, or 250 ml to 500 ml. The kit may optionally also comprise instructions. These instructions preferably regard the use of the containers and compositions) of the kit.

Further described herein is a method for treating a subject/patient, comprising administering to said subject/patient in an amount and by a route effective for treatment, a composition obtained by the methods according to the present invention.

Any one of the compositions described herein are for use as a medicament.

Any one of the compositions described herein or the kit described herein are for use in a method for the treatment of a patient suffering from any disease, preferably cancer.

Also described herein is a method for preparing a composition comprising a protein, preferably an antibody or antibody fragment or Fc-fusion protein, at a concentration of at least 100 mg/ml, wherein said composition does not contain a buffer excipient, comprising removing residual buffer excipients, preferably via dialysis or ultrafiltration/diafiltration (UF/DF).

The invention also relates to the following items:

1. A method of administrating a composition to a subject comprising the following steps:
a) Providing a composition comprising a protein, preferably an antibody or antibody fragment or Fc-fusion protein, at a concentration of at least 100 mg/ml, wherein said composition does not contain a buffer excipient,

b) Diluting said composition of step a) into a larger volume of a solution,

c) Administrating said solution of step b) to a subject.

2. A method of preparing a composition for administration to a subject comprising the following steps:

a) Providing a composition comprising a protein, preferably an antibody or antibody fragment or Fc-fusion protein, at a concentration of at least 100 mg/ml, wherein said composition does not contain a buffer excipient,

b) Diluting said composition of step a) into a larger volume of a solution.

3. A kit comprising in one or more containers

a) a composition according to the invention comprising a protein, preferably an antibody or antibody fragment or Fc-fusion protein, at a concentration of at least 100 mg/ml, wherein said composition does not contain a buffer excipient, having a certain volume, and

b) a larger volume of a solution according to the invention.

4. A method or a kit according to any one of items 1 to 3, wherein the composition comprising a protein at a concentration of at least 100 mg/ml as described under a), has a volume of 0.1 ml to 20 ml.

5. A method or a kit according to any one of items 1 to 4, wherein the composition comprising a protein at a concentration of at least 100 mg/ml as described under a), has a volume of 1 ml to 10 ml.

6. A method or a kit according to any one of items 1 to 5, wherein the composition comprising a protein at a concentration of at least 100 mg/ml as described under a), has a volume of 1 ml.

7. A method or a kit according to any one of items 1 to 6, wherein the larger volume of a solution under b), is between 50 ml to 1 L.

8. A method or a kit according to any one of items 1 to 7, wherein the larger volume of a solution under b), is between 100 ml to 500 ml.
9. A method or a kit according to any one of items 1 to 8, wherein the larger volume of a solution under b), is 250 ml.
10. A method or a kit according to any one of items 1 to 9, wherein the larger volume of a solution under b), is 500 ml.

11. A method or a kit according to any one of items 1 to 10, wherein the larger volume of a solution under b), is 250 ml to 500 ml.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of medicine, pharmacy, chemistry, biology, oncology, cell biology, molecular biology, cell culture, immunology and the like which are in the skill of one in the art. These techniques are fully disclosed in the current literature.

The following examples are not limiting. They merely show possible embodiments of the invention. A person skilled in the art could easily adjust the conditions to apply it to other embodiments.

EXPERIMENTAL
MATERIALS AND METHODS
Four monoclonal antibodies (mAb1, mAb2, mAb3 and mAb4) used in this study were produced by mammalian cell culture technology (Bergeman2007). Base and acid standard solutions (1 N or 0.1 N HCl and accordingly 1 N or 0.1 N NaOH; volumetric) used for the titration studies were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solutions were prepared using water for injection (WFI).

The buffer systems being investigated in terms of buffer capacity were acetate, citrate, succinate, phosphate and histidine buffers. Chemicals used in the preparation thereof were of analytical grade. The buffers were prepared at concentrations of 10, 25 and 50 mM. To simulate conditions close to the situation in the development of sub-cutaneous formulations, the solutions also contained 130 mM NaCl (acetate buffer), 125 mM NaCl (citrate and succinate buffer), 135 mM NaCl (phosphate), 140 mM NaCl (histidine buffer). These saline contents each plus the contribution of 25 mM buffer salt adjust the solutions to isotonicity.

Antibody solutions were prepared as follows. For antibodies mAb1 -3 the bulk was first ultrafiltered over a tangential flow filtration system (Sartorius, Goettingen, Germany) to a
final concentration of approximately 100 mg/ml. In a second step, the concentrated solution was diafiltered against a solution containing 160 mM NaCl (or 10 mM as indicated). Here, the minimum of diavolume exchanges was at least eight exclusion volumes. Thereby, a practically complete replacement of the antibody storage buffer is ensured since, in theory, after eight diafiltration volumes at a constant protein concentration, the solution is up to 99.96 % diafiltered in the new solution if the membrane is 100 % permeable to small molecules (ZYndey2000). Protein dilutions were prepared in 160 mM NaCl (or 10 mM as indicated). mAb4 was ultrafiltered against water for injection obtaining a protein concentration > 200 mg/ml. The solution was then adjusted to isotonicity (~ 300 mosmol/kg) by spiking with NaCl or trehalose (as indicated). Dilutions of mAb4 were prepared with aqueous NaCl or trehalose retaining around 300 mosmol/kg of the solution being titrated. Final protein concentrations of the mAb solutions were determined via their absorption at λ = 280 nm employing the mAb specific extinction coefficients.

Titration curves were generated by adding HCl or NaOH standard solutions to 5 ml of each buffer system in a clean IOR-glass vial (Fiolax Clear Glass Vials, Schott forma vitrum, Mullheim, Germany), mixing and measuring the pH value (WTW pH 340, Wissenschaftlich-Technische Werkstatten, Weilheim, Germany). The pH meter had been calibrated via a two point calibration using standard buffer solutions pH 4.0 and 7.0 (Mettler-Toledo, Schwerzenbach, Switzerland). Titrations were performed starting from pH 6.0 to pH 4.0 or to pH 8.0 respectively. The concentration of the added standard solution (0.1 or 1 N) to change the pH in reasonable increments had been determined in preliminary tests. The titration time was kept as low as possible to minimize the CO₂-input and the interaction of the solution with the glass vial. All pH measurements were performed at 5 °C, 25 °C or 40 °C as indicated and the pH value was recorded after the value had stabilized for at least 10 seconds.

For evaluation, the pH was plotted versus the microequivalents $^\mu$Eq of acid or base added for each titration normalized for the solution volume. For monoprotic and monobasic compounds, such as HCl and NaOH, the $^\mu$Eq is equal to the micromoles of titrant added. Titration curves were dissected in segments and the data points of each segment were fitted to a linear equation. The inverse value of the slope yields the average buffer capacity β for the
chosen pH range. The buffer capacity describes how many microequivalents can be added to 1 ml of the buffer or antibody system before changing the pH one unit.

Finally, the buffer capacity of a certain buffer system was plotted versus buffer concentration and the linear fit thereof yields the 'slope of capacity'. Employing the 'slope of capacity', buffer capacities of different buffer systems can be compared and the buffer capacities at further concentrations can be extrapolated.

For theoretical determination of the buffer capacity the equation of van Slyke was used. According to van Slyke the buffer capacity ($\beta$) of an aqueous solution is described by equations (1) and (2) (vanSlykel922).

$$
\beta = 2.3 \left( \frac{K_a C[H^+]}{[K_a + [H^+]]^2} + [H^+] + [OH^-] \right)
$$

$$
\beta = 2.3 C[H^+] \left( \frac{K_{a1}}{[K_{a1} + [H^+]]^2} + \frac{K_{a2}}{[K_{a2} + [H^+]]^2} + \ldots \right) + 2.3 ([H^+] + [OH^-])
$$

Here, $C$ is the total buffer concentration and $K_a$ is the acid constant of the buffer. Equation 1 is employed to buffer systems that are characterized by a single pKa value only. More complex systems are described by equation 2 adding up various pKa values to a total buffer capacity.

As an example, the effect of three ionizable groups in citrate and histidine on the total buffer capacity is depicted in Figure 1A and 1B respectively. In citrate the pKa values of three carboxyl groups are around 1 unit apart leading to a constantly large buffer capacity over a broad range (pH 2.5-6.0) (Figure 1A). In contrast, the pKa values of the amino group, the carboxyl group and the imidazole moiety in histidine differ up to 4 units. Thus, the buffer effects of these groups hardly overlap and significant buffer capacity is found in narrow pH ranges only (Figure 1B).

Equation 2 yields the buffer capacity for discrete pH values. Therefore, the calculated average value within $\Delta \rho H = 1$ or 0.5 (as indicated) was compared with the experimentally determined value for the same range.

For calculation of the buffering capacity of an antibody equation 3 has been suggested by Gokarn and colleagues (Gokarn2008).

$$
\beta_{mAb} = \sum_{i=Aq} N_i \cdot C_{mAb} \cdot \left\{ \left[ \frac{[H^+]}{[K_a]_i + [H^+]} \right]_{\rho pH} - \left[ \frac{[H^+]}{[K_a]_i + [H^+]} \right]_{\rho pH+1} \right\} + ([H^+]_{\rho pH} - [H^+]_{\rho pH+1})
$$
In equation 3, the subscript $i$ refers to the amino acids aspartic acid, glutamic acid and histidine (relevant for the buffering within the pH range of interest), $N$ is the number of amino acids of the mAb, $C_{mAb}$ is the molar concentration of mAb and $K_a$ is the acid dissociation constant of the corresponding amino acid side-chain. The indices "pH" and "pH+1" denote the start and end value of the specific pH range $\Delta \rho H = 1$ being addressed.

Table 1 summarizes the pKa values used for the calculations and Table 2 the number of the amino acids in niAbs1-3 contributing to the buffer capacity in the pH range of relevance.

Table 1: pKa values used for calculating theoretical buffer capacities (Lide2004 (at zero ionic strength, 25 °C), Hastingsl922; Thurlkill2006a (0.1 M KC1 at 25 °C))

<table>
<thead>
<tr>
<th>Buffer</th>
<th>pKa1</th>
<th>pKa2</th>
<th>pKa3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>4.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td>3.08</td>
<td>4.39</td>
<td>5.49</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.8</td>
<td>6.04</td>
<td>9.33</td>
</tr>
<tr>
<td>Succinate</td>
<td>4.21</td>
<td>5.64</td>
<td></td>
</tr>
<tr>
<td>Phosphate</td>
<td>2.15</td>
<td>7.20</td>
<td>12.35</td>
</tr>
</tbody>
</table>

Side chain of

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>His</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>Asp</td>
<td>3.7</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Number of histidine, aspartate and glutamate residues in the monoclonal antibodies assessed in the present study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>His</th>
<th>Asp</th>
<th>Glu</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAb1</td>
<td>24</td>
<td>48</td>
<td>68</td>
</tr>
<tr>
<td>mAb2</td>
<td>24</td>
<td>60</td>
<td>58</td>
</tr>
<tr>
<td>mAb3</td>
<td>26</td>
<td>50</td>
<td>72</td>
</tr>
</tbody>
</table>
**EXAMPLES**

**EXAMPLE 1: BUFFER CAPACITY OF STANDARD BUFFERS**

To determine the buffer capacity $\beta$ of acetate, citrate, succinate, phosphate and histidine, the corresponding buffer solutions were titrated with acid or base and the resulting pH was measured. Figure 2 summarizes the titration of citrate buffer as a representative result. In the range of pH 4.0-6.0 and pH 6.0-6.5 a fairly linear dependence of pH and $\mu$Eq is observed. For pH values greater 6.5 the buffer capacity of citrate is comparably low (cf. Figure 1A) and further addition of base leads to drastic pH shifts.

As a matter of fact, the buffer capacity can only be determined for a discrete value by differentiating pH value with respect to acid/base equivalents added (equation 4, exemplarily $B$ for base equivalents).

$$\beta = \frac{dB}{d\pi}$$

However, the average buffer capacity over a limited pH range (equation 5) serves as an acceptable approximation (vanSlyke922) and is moreover usually of practical interest.

$$\beta \approx \frac{AB}{\Delta pH}$$

Therefore an averaged buffer capacity is derived from the linear fit of the data points within roughly linear segments of the titration curve of $\Delta p$H = 1 (in few cases we chose $\Delta p$H = 0.5 when the titration curve was insufficiently described by a linear curve within $\Delta p$H =1). This yields average buffer capacities for the assayed buffer solutions in defined pH ranges (cf. Table 3).

<table>
<thead>
<tr>
<th>buffer system</th>
<th>pH-range</th>
<th>experiment</th>
<th>model of van Slyke</th>
<th>ratio of slopes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\mu$Eq/$\mu$mol</td>
<td>$\mu$Eq/$\mu$mol</td>
<td>$a_{exp}$/$a_{vanSlyke}$</td>
</tr>
<tr>
<td>citrate</td>
<td>4.0-5.0</td>
<td>0.78</td>
<td>0.82</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>5.0-6.0</td>
<td>0.60</td>
<td>0.70</td>
<td>0.87</td>
</tr>
<tr>
<td>Buffer Excipient</td>
<td>6.0-6.5</td>
<td>6.0-7.0</td>
<td>7.0-8.0</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>histidine</td>
<td>0.31</td>
<td>0.49</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>acetate</td>
<td>0.04</td>
<td>0.47</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>succinate</td>
<td>0.50</td>
<td>0.64</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>phosphate</td>
<td>0.33</td>
<td>0.47</td>
<td>0.39</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 summarizes the experimentally derived parameters for the buffer excipients acetate, citrate, succinate, phosphate and histidine which are widely used in protein science. The parameters allow for the computation of the average buffer capacity for these buffers in a certain pH range at any desired concentration by multiplying parameter \( a \) with the buffer concentration of interest. This procedure is successfully applied in our lab. It allows for an estimate how much acid/base needs to be added to a buffered solution of known concentration to achieve a certain pH shift.
In addition, Table 3 lists the corresponding parameters for values simulated employing the van Slyke equation. Except for phosphate buffer, we find a good correlation between experimentally derived values and predicted values. Larger deviations can be seen for pH ranges in which the corresponding buffer exhibits comparably low buffer capacity (pH far from pKa). Here, a larger susceptibility of the parameters to experimental errors is anticipated.

EXAMPLE 2: BUFFER CAPACITY OF MONOCLONAL ANTIBODIES

To determine the buffer capacity $\beta$ of monoclonal antibodies, antibody solutions in 160 mM NaCl were titrated with acid or base and pH values were recorded. Titration curves of mAb3 are exemplarily shown in Figure 4. The ranges of pH 6.0-5.0, 5.0-4.0 and 6.0-7.0 are plotted here and the titration curves are representative for all monoclonal antibodies used in this study since we found no markedly significant differences between the studied antibodies. We point out that a significant contribution by residual buffering components from the bulk formulation of the mAbs to the buffer capacity can be excluded since an eightfold (by volume) exchange to 160 mM NaCl had been performed in preparation of the solutions (methods section).

Within pH ranges of $\Delta$H=1, we find a practically linear correlation of the amount of base/acid added and the resulting pH just as described for the conventional buffers (previous section). Data points were fitted to linear equations and the derived buffer capacities $\beta$ scale linearly with the protein concentration equally as shown for the buffer systems in the previous section (Figure 5).

When titrating with NaOH (pH 6.0-7.0) a steeper slope corresponding to a lower buffer capacity than in the titration with HCl (pH 4.0-6.0) is observed. This effect reflects the amount of amino acids buffering in the range of pH 4.0-7.0. As listed in Table 2, the side chains of 50 aspartic acids ($pK_a=3.7$) and 72 glutamic acids ($pK_a=4.3$) are capable of buffering up to about pH 5.5 whereas only 26 histidine side chains ($pK_a=6.5$) contribute to the buffer capacity in the range of pH 5.5-7.5. Similar proportions can be found for mAb1 and mAb2.

In Figure 5 the experimentally determined buffer capacities are plotted versus the concentration of mAb1, mAb2 and mAb3. Since the buffer capacity increases linearly with
concentration, just as found for conventional buffers, the data points were fitted to a linear equation. The slope i.e. the slope of capacity was determined and turns out to be nearly identical for mAb1, mAb2 and mAb3. This similarity was found for the overall pH-range of 4.0-8.0 (cf. Table 4).

Table 4: Experimentally and theoretically derived slopes of capacity (a) for monoclonal antibodies mAb1, mAb2 and mAb3 at various pH ranges:

<table>
<thead>
<tr>
<th>pH-range</th>
<th>experiment</th>
<th>ab initio prediction</th>
<th>ratio of slopes</th>
<th>model of van Slyke</th>
<th>ratio of slopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 4.0-5.0</td>
<td>µEq/mg</td>
<td>µEq/mg</td>
<td>µEq/mg</td>
<td>µEq/mg</td>
<td></td>
</tr>
<tr>
<td>mAb1</td>
<td>0.183</td>
<td>0.354</td>
<td>0.52</td>
<td>0.350</td>
<td>0.52</td>
</tr>
<tr>
<td>mAb2</td>
<td>0.160</td>
<td>0.322</td>
<td>0.49</td>
<td>0.320</td>
<td>0.50</td>
</tr>
<tr>
<td>mAb3</td>
<td>0.203</td>
<td>0.332</td>
<td>0.61</td>
<td>0.329</td>
<td>0.62</td>
</tr>
<tr>
<td>pH 5.0-6.0</td>
<td>µEq/mg</td>
<td>µEq/mg</td>
<td>µEq/mg</td>
<td>µEq/mg</td>
<td></td>
</tr>
<tr>
<td>mAb1</td>
<td>0.084</td>
<td>0.126</td>
<td>0.67</td>
<td>0.129</td>
<td>0.65</td>
</tr>
<tr>
<td>mAb2</td>
<td>0.080</td>
<td>0.111</td>
<td>0.72</td>
<td>0.113</td>
<td>0.70</td>
</tr>
<tr>
<td>mAb3</td>
<td>0.097</td>
<td>0.117</td>
<td>0.82</td>
<td>0.119</td>
<td>0.81</td>
</tr>
<tr>
<td>pH 6.0-7.0</td>
<td>µEq/mg</td>
<td>µEq/mg</td>
<td>µEq/mg</td>
<td>µEq/mg</td>
<td></td>
</tr>
<tr>
<td>mAb1</td>
<td>0.055</td>
<td>0.104</td>
<td>0.52</td>
<td>0.103</td>
<td>0.53</td>
</tr>
<tr>
<td>mAb2</td>
<td>0.042</td>
<td>0.095</td>
<td>0.44</td>
<td>0.093</td>
<td>0.45</td>
</tr>
<tr>
<td>mAb3</td>
<td>0.048</td>
<td>0.095</td>
<td>0.50</td>
<td>0.094</td>
<td>0.50</td>
</tr>
<tr>
<td>pH 7.0-8.0</td>
<td>µEq/mg</td>
<td>µEq/mg</td>
<td>µEq/mg</td>
<td>µEq/mg</td>
<td></td>
</tr>
<tr>
<td>mAb1</td>
<td>0.051</td>
<td>0.039</td>
<td>1.31</td>
<td>0.039</td>
<td>1.29</td>
</tr>
<tr>
<td>mAb2</td>
<td>0.031</td>
<td>0.036</td>
<td>0.86</td>
<td>0.036</td>
<td>0.84</td>
</tr>
<tr>
<td>mAb3</td>
<td>0.043</td>
<td>0.036</td>
<td>1.20</td>
<td>0.036</td>
<td>1.18</td>
</tr>
</tbody>
</table>
the slope of capacity was derived from simulations employing a model suggested by Gokarn et al., equation 3 (Gokarn 2008).

The graphs in Figure 5A and 5B also include the experimentally determined buffer capacities of the conventional buffer citrate and histidine respectively at different concentrations in the pH-range pH 6.0-5.0 (secondary axis). We find that the monoclonal antibodies exhibit inherent buffer capacity that at 50 mg/ml resembles the capacity of 6 mM citrate or 14 mM histidine buffer in the pH-range of pH 6.0-5.0. As 10 mM histidine is a commonly used buffer (e.g. end-formulation of Rituximab (Wang 2007)), we conclude the self-buffering effect of a 50 mg/ml antibody solution is sufficient to replace the buffer excipient.

Table 4 additionally lists the parameters for mAb1, mAb2 and mAb3 determined via the theoretical models (equations 2 and 3). Here, the buffer capacities of all buffering amino acids (cf. Tables 1, 2) are regarded as independently summing up to a total buffer capacity. We find deviations in the experimentally determined parameters and the theoretical values. On average the slopes of capacity differ by a factor of 2. This might be due to the fact that the pKa values of the amino acid side chains can shift in the native protein environment compared to the theoretical pKa values for the ionizable side chain in isolation. As a result, they buffer in a pH range different from the predicted. We therefore suggest that the buffering characteristics should be derived experimentally for the antibody of interest.

EXAMPLE 3: BUFFER CAPACITY OF A MONOCLONAL ANTIBODY AT THE HIGH-CONCENTRATION REGIME

Recently, pharmaceutical development is heading towards protein solutions of very high concentrations. We therefore also characterized an antibody solution of more than 200 mg/ml protein in terms of buffer capacity. To approximate a real-life situation, we titrated samples containing antibody plus a non-buffering excipient that adjusts the osmolality of the solutions to around 300 mosmol/kg (i.e. close to isotonicity). Figures 6 and 7 summarize the titration curves for mAb4 plus NaCl and trehalose respectively at different protein concentrations.
including 220 mg/ml. We find adequate linearity of the titration curves for ΔpH= 1 in the range of pH 4.0-7.0. The buffer capacities for certain pH ranges were plotted versus protein concentration (exemplarily in figure 8) and the slope of capacity calculated.

Table 5: Experimentally determined slopes of capacity for different pH ranges and mAb4 in a trehalose or NaCl formulation

<table>
<thead>
<tr>
<th>pH-range</th>
<th>slope of capacity (μEq/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0-4.0</td>
<td>In trehalose</td>
</tr>
<tr>
<td></td>
<td>In NaCl</td>
</tr>
<tr>
<td>6.0-5.0</td>
<td>0.083</td>
</tr>
<tr>
<td>6.0-7.0</td>
<td>0.048</td>
</tr>
<tr>
<td>7.0-8.0</td>
<td>0.046</td>
</tr>
</tbody>
</table>

Table 5 summarizes the corresponding values from titrations of antibody solutions containing NaCl or trehalose. We find a linear increase of buffer capacity with increasing protein concentration up to more than 200 mg/ml under conditions close to isotonicity. In the pH range of pH 4.0-5.0 the antibody solution of 220 mg/ml protein in trehalose or NaCl exhibits a buffer capacity resembling the capacity of around 60 mM citrate buffer. In the range of pH 5.0-6.0 this highly concentrated antibody solution buffers like 50 mM histidine or 30 mM citrate buffer. These values underline that the buffer capacity of the protein can substitute for a buffering excipient in highly concentrated antibody solutions.

EXAMPLE 4: BUFFER CAPACITY OF ANTIBODIES AT VARYING TEMPERATURE
During biopharmaceutical development antibody solutions can be exposed to different temperatures. For example, antibodies are commonly stored and shipped at 2-8 °C and for accelerated high temperature stability studies temperatures up to 40 °C are chosen. As any equilibrium constant (and therefore the pKa) varies with temperature, the buffer capacity in a defined pH range is also subject to change. For proteins, a temperature change has further consequences (e.g. changing conformational equilibria) and an estimation of the total effect of a temperature shift on the self-buffering characteristics of proteins is not straight-forward. To, therefore, check whether the antibody inherent buffer capacity might be able to prevent pH shifts
also at varying temperature, we set out to determine the effect of temperature on buffer capacity experimentally. Figure 9 depicts titration curves of mAb3 at 5 °C, room temperature and 40 °C each at different protein concentration. Control experiments employing differential scanning calorimetry showed the antibody in 160 mM NaCl is fully folded at 40 °C. The first unfolding event takes place at temperatures above ~ 60 °C (Tml = 67 °C, data not shown).

In the titration curves at 5 °C, 25 °C and 40 °C we find a linear dependence of pH value and added µEq of acid in the range of pH 4.0-5.0 and pH 5.0-6.0 at all temperatures. Moreover, the slope of the curve and hence the buffer capacity is nearly identical for all temperatures. This holds true for titrations at protein concentrations of 10 mg/ml and 100 mg/ml. Also, in the range of pH 6.0-7.0 and pH 7.0-8.0 a nearly identical buffer capacity was determined from titrations at 5 °C, room temperature and 40 °C (data not shown). The experiments indicate the inherent buffer capacity of mAbs is not significantly altered upon temperature changes within the range relevant for biopharmaceutic processes.

EXAMPLE 5: BUFFER CAPACITY OF ANTIBODIES AND A CONVENTIONAL BUFFER AT VARYING IONIC STRENGTH

According to the Debye Huckel theory (Debyel923) the activity coefficients of the species in an ionization equilibrium and therefore the pKa of acids depend on the ionic strength of a solution. A change in ionic strength can thus alter the average buffer capacity of a defined pH range. To check for the practical relevance of this effect, we determined average buffer capacities of buffers for different ionic strength conditions. The titration curves for mAb3 at different ionic strength can be found in Figure 10. We find that a change in ionic strength in the range of 10 mM NaCl to 160 mM NaCl (commonly employed in mAb formulations) has a moderate effect on the buffer capacity of mAb3 in the range of pH 5.0-6.0 and pH 6.0-7.0 (change in β around 35 % and 25 % respectively). For the conventional buffer histidine the effect is rather negligible (change in β below 15 % and 10 % respectively).
EXAMPLE 6: PREPARATION OF A DILUTED MONOCLONAL ANTIBODY SOLUTION FOR INFUSION FROM A CONCENTRATE OF THE MONOCLONAL ANTIBODY

The monoclonal antibody (mAb) solution of more than 110 mg/ml not containing buffer excipient is used for storage. A solution for infusion is prepared by diluting this solution into physiological salt solution in an infusion bag resulting in pH values of the composition as tabulated.

<table>
<thead>
<tr>
<th>Concentration mAb in concentrate</th>
<th>Concentration mAb in infusion bag [mg/ml]</th>
<th>pH in infusion bag</th>
<th>Concentration mAb in infusion bag [mg/ml]</th>
<th>pH in infusion bag</th>
</tr>
</thead>
<tbody>
<tr>
<td>136 mg/ml</td>
<td>0.1</td>
<td>5.2</td>
<td>0.1</td>
<td>5.65</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>4.98</td>
<td>0.5</td>
<td>6.10</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4.92</td>
<td>1</td>
<td>6.28</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.89</td>
<td>2</td>
<td>6.54</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.86</td>
<td>5</td>
<td>6.67</td>
</tr>
</tbody>
</table>

From the same stock solution of a mAb a concentrate of pH 4.67 was prepared by titration with HCl and a concentrate of pH 6.67 was prepared by titration with NaOH.

In a first series of experiments a first concentrate of the mAb which had a pH of 4.67 was diluted to the concentration of mAb indicated in the table with a physiological salt solution in an infusion bag. The volume of the infusion bag was 50 ml.

In a second series of experiments a second concentrate of the mAb which had a pH of 6.67 was diluted in the same manner.

Notably, in the presence of mAb the pH in the infusion bag was different from the apparent pH of the physiological salt solution in the infusion bag, which was pH 5.5. The data demonstrate that, in the absence of a buffer in the infusion bag, the pH of the solution for infusion varies depending on the concentration of mAb in the solution for infusion. Therefore, the concentration of mAb in the solution for infusion may be selected such as to provide the optimal pH for the mAb. The data shown here illustrate the procedure according to the methods of the invention for preparing a composition for administration to a subject, e.g., by infusion, wherein an isotonic solution compatible with administration to a patient was used for the dilution. Even at high ionic strength of around 160mM NaCl the mAb has a significant buffering capacity, which may be
used to adjust the pH of the solution for infusion, especially in the range of pH 4.0 to 7.0, and more particularly, in the range of pH 4.0 to 6.0 or in the range of pH 5.0 to 7.0.

**LITERATURE CITED**


45


(41) Van Slyke DD. On the measurement of buffer values and on the relationship of buffer value to the dissociation constant of the buffer and the concentration and the reaction of the buffer solution. J Biol Chem 1922 June 1;52(2):525-70.


Claims

1. A method of administrating a composition to a subject comprising the following steps:
   a) Providing a composition comprising a protein, preferably an antibody or antibody fragment or Fc-fusion protein, at a concentration of at least 100 mg/ml, wherein said composition does not contain a buffer excipient, preferably having a volume of 0.1 ml to 20 ml, or 1 ml to 10 ml, preferably 1 ml,
   b) Diluting said composition of step a) into a larger volume of a solution, wherein the larger volume is between 50 ml to 1 L, or between 100 ml to 500 ml, preferably 250 ml, or 500 ml, or 250 ml to 500 ml,
   c) Administrating said solution of step b) to a subject.

2. A method of preparing a composition for administration to a subject comprising the following steps:
   a) Providing a composition comprising a protein, preferably an antibody or antibody fragment or Fc-fusion protein, at a concentration of at least 100 mg/ml, wherein said composition does not contain a buffer excipient, preferably having a volume of 0.1 ml to 20 ml, or 1 ml to 10 ml, preferably 1 ml,
   b) Diluting said composition of step a) into a larger volume of a solution, wherein the larger volume is between 50 ml to 1 L, or between 100 ml to 500 ml, preferably 250 ml, or 500 ml, or 250 ml to 500 ml.

3. The method according to claim 1 or 2, wherein the larger volume of a solution is an isotonic solution, such as 0.9% m/V NaCl in water, preferably with an osmolality of around 300 mosmol/kg (osmolarity of around 300 mosmol/l).

4. The method according to any one of claims 1 to 3, wherein said composition comprising a protein at a concentration of at least 100 mg/ml, is an isotonic solution which has an osmolality of around 300 mosmol/kg (osmolarity of approximately 300 mosmol/l).

5. The method according to any one of claims 1 to 4, wherein said composition comprising a protein at a concentration of at least 100 mg/ml, is an aqueous solution, preferably an isotonic solution such as 0.9% m/V NaCl in water.

6. The method according to any one of claims 1 to 3, wherein said composition comprising a protein at a concentration of at least 100 mg/ml is non-isotonic.
7. The method according to any one of claims 1 to 6, wherein the protein provides 100% of the buffer capacity of the composition comprising a protein at a concentration of at least 100 mg/ml.

8. The method according to any one of claims 1 to 7, wherein in the composition comprising a protein at a concentration of at least 100 mg/ml the only other component besides the protein component is water (H₂O), preferably water for injection (WFI).

9. The method according to any one of claims 1 to 8, wherein the protein is a therapeutic protein, preferably a therapeutic antibody, an antibody fragment, an Fc-fusion protein, a full-length immunoglobulin molecule, an immunoglobulin based molecule such as an immunoglobulin fragment, an immunoglobulin isoform, a fusion protein comprising at least one immunoglobulin chain, or an immunoglobulin conjugated to a non-proteinaceous moiety.

10. The method according to any one of claims 1 to 9, wherein the protein concentration is at least 110 mg/ml, at least 115 mg/ml, at least 120 mg/ml, at least 150 mg/ml, at least 200 mg/ml, at least 220 mg/ml, at least 300 mg/ml or within the range of 100 mg/ml to 220 mg/ml or 100 mg/ml to 300 mg/ml.

11. The method according to any one of claims 1 to 10, wherein at the pH of the composition, 25°C, one atmosphere, and equilibrium with ambient atmosphere, the protein has a buffer capacity of at least that of approximately 4.0 mM citrate buffer in the pH range of pH 4.0 to 7.0 under the same conditions.

12. The method according to any one of claims 1 to 10, wherein at the pH of the composition, 25°C, one atmosphere, and equilibrium with ambient atmosphere, the protein has a buffer capacity of approximately 60 mM citrate buffer in the pH range of pH 4.0 to 5.0 under the same conditions.

13. The method according to any one of claims 1 to 10, wherein at the pH of the composition, 25°C, one atmosphere, and equilibrium with ambient atmosphere, the protein has a buffer capacity of approximately 50 mM histidine or 30 mM citrate buffer in the pH range of pH 5.0 to 6.0 under the same conditions.
14. The method according to any one of claims 1 to 13, wherein the pH of the composition 
maintained by the buffering action of the protein is between approximately pH 4.0 and pH 
8.0, preferably pH 4.0 to pH 7.0, or pH 4.0 to pH 6.0, or pH 5.0 to 7.0.
15. The method according to any one of claims 1 to 14, wherein the pH of the composition 
maintained by the buffering action of the protein is pH 4.0 to pH 5.0, pH 5.0 to pH 6.0, or 
pH 6.0 to pH 7.0.
16. The method according to any one of claims 1 to 15, wherein the composition comprising a 
protein at a concentration of at least 100 mg/ml is provided by reconstitution of a 
lyophilisate.
17. The method according to any one of claims 1 to 16, which comprises removing residual 
buffer excipients, preferably via dialysis or ultrafiltration/diafiltration (UF/DF), for 
preparing the composition comprising a protein at a concentration of at least 100 mg/ml.
18. The method according to any one of the preceding claims, wherein one or more further 
proteins are added as a separate composition prior to or subsequently to diluting said 
composition of step a) into a larger volume of a solution, or as part of the larger volume of 
a solution used for the dilution of the composition of step a).
19. The method according to claim 18, wherein the one or more further proteins are selected 
from the group consisting of a therapeutic protein, preferably a therapeutic antibody, an 
antibody fragment, an Fc-fusion protein, a full-length immunoglobulin molecule, an 
immunoglobulin based molecule such as an immunoglobulin fragment, an 
immunoglobulin isoform, a fusion protein comprising at least one immunoglobulin chain, 
or an immunoglobulin conjugated to a non-proteinaceous moiety.
20. The method according to claims 18 or 19, wherein the one or more further proteins are 
added as a separate composition comprising the one or more further proteins at a (total) 
concentration of at least 100 mg/ml.
21. The method according to any one of claims 18 to 20, wherein the one or more further 
protein provide/s 100% of the buffer capacity of the separate composition comprising the 
one or more further proteins.
22. The method according to any one of claims 18 to 21, wherein the (total) concentration of 
the one or more further proteins in the separate composition is at least 110 mg/ml, at least
115 mg/ml, at least 120 mg/ml, at least 150 mg/ml, at least 200 mg/ml, at least 220 mg/ml, at least 300 mg/ml or within the range of 100 mg/ml to 220 mg/ml or 100 mg/ml to 300 mg/ml.

23. The method according to any one of the preceding claims, wherein the protein, or the protein and the one or more further proteins, provide/s 100% of the buffer capacity of the solution obtained in step b).

24. A kit comprising in one or more containers a composition comprising a protein, preferably an antibody or antibody fragment or Fc-fusion protein, at a concentration of at least 100 mg/ml, wherein said composition does not contain a buffer excipient, having a volume of 0.1 ml to 20 ml, or 1 ml to 10 ml, preferably 1 ml, and a larger volume of a solution wherein the larger volume is between 50 ml to 1 L, or between 100 ml to 500 ml, preferably 250 ml, or 500 ml, or 250 ml to 500 ml.

25. The kit of claim 24, additionally comprising instructions.
FIGURES

FIGURE 1

A

B

buffer capacity (μEq/ml)

pKₐ₁, pKₐ₂, pKₐ₃

buffer capacity (μEq/ml)

pKₐ₁, pKₐ₂, pKₐ₃

pH

pH

FIGURE 2

A

B

μEq/ml of HCl

μEq/ml of NaOH

pH

pH

10 mM citrate

25 mM citrate

50 mM citrate

10 mM citrate

25 mM citrate

50 mM citrate

-1-
FIGURE 3

\[ \beta(x)_{\text{pH y-z}} = a \cdot c_x \]

\[ \beta(\text{citrate})_{\text{pH 6.0-5.0}} = 0.60 \cdot c_{\text{citrate}} \]

Citrate concentration / mM

FIGURE 4

A

B

-2-
FIGURE 7

A

\[ \text{pH} \]

\[ \text{\(\mu\text{Eq/ml of HCl}\)} \]

\[ \text{\(\Delta 220 \text{ mg/ml mAb}\)} \]

\[ \bullet 100 \text{ mg/ml mAb}\]

\[ \text{■ 24 mg/ml mAb}\]

B

\[ \text{pH} \]

\[ \text{\(\mu\text{Eq/ml of NaOH}\)} \]

\[ \text{\(\Delta 220 \text{ mg/ml mAb}\)} \]

\[ \bullet 100 \text{ mg/ml mAb}\]

\[ \text{■ 24 mg/ml mAb}\]
FIGURE 8

\[
\begin{align*}
\beta (5.0-4.0) & \quad /\mu\text{Eq/ml} \\
\text{mAb concentration / mg/ml} & \\
\end{align*}
\]

- mAb + Trehalose
- mAb + NaCl

FIGURE 9

A

100 mg/ml • at 25 °C
• at 40 °C
▲ at 5 °C
10 mg/ml □ at 25 °C
□ at 40 °C
▲ at 5 °C

pH

\[
\begin{align*}
\mu\text{Eq/ml of HCl} & \\
\end{align*}
\]

B

100 mg/ml • at 25 °C
• at 40 °C
▲ at 5 °C
10 mg/ml □ at 25 °C
□ at 40 °C
▲ at 5 °C

pH

\[
\begin{align*}
\mu\text{Eq/ml of HCl} & \\
\end{align*}
\]
FIGURE 10

A

<table>
<thead>
<tr>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
</tr>
<tr>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>μEq/ml of HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
</tbody>
</table>

- □ 50 mg/ml mAb + 160 mM NaCl
- • 50 mg/ml mAb + 10 mM NaCl
- □ 25 mM histidine + 140 mM NaCl
- ○ 25 mM histidine + 10 mM NaCl

B

<table>
<thead>
<tr>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0</td>
</tr>
<tr>
<td>6.75</td>
</tr>
<tr>
<td>6.5</td>
</tr>
<tr>
<td>6.25</td>
</tr>
<tr>
<td>6.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>μEq/ml of NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
</tbody>
</table>

- □ 50 mg/ml mAb + 160 mM NaCl
- • 50 mg/ml mAb + 10 mM NaCl
- □ 25 mM histidine + 140 mM NaCl
- ○ 25 mM histidine + 10 mM NaCl
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61K38/00 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, EMBASE, CHEM ABS Data, BIOSIS, Sequence Search

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>WO 2012/013980 AI (JEZEK JAN [GB]; DERHAM BARRY KINGSTON [GB]; ZAPADKA KAROLINA [GB]; ARE) 2 February 2012 (2012-02-02) page 9, lines 1-5 page 7, lines 13-21 claims 1-9,29-37,42</td>
<td>1-25</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document(s) which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

<table>
<thead>
<tr>
<th>Date of the actual completion of the international search</th>
<th>Date of mailing of the international search report</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 October 2013</td>
<td>16/10/2013</td>
</tr>
</tbody>
</table>

Name and mailing address of the ISA:

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, 340-3016

Authorized officer

Greff, Gabri et a
<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>DE 10.2006 024528 AI (ALBUPHARM HEIDELBERG GMBH &amp; CO [DE])</td>
<td>1-25</td>
</tr>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-----------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>US 2008311078 Al</td>
<td>18-12-2008</td>
<td>AU 2006259664 Al</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BR PI0611901 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2610839 Al</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 101217979 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EA 200800050 Al</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 1909831 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2008543839 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2013047261 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KR 20080031684 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SG 162788 Al</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2008311078 Al</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2012028877 Al</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2006138181 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZA 2008003338 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 2598167 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2013209465 Al</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2012013980 Al</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DE 102006024528 Al</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DE 102006024528 Al</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 2026789 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2007134595 A2</td>
</tr>
</tbody>
</table>