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(54) Title: SELF-BUFFERING PROTEIN FORMULATIONS

(57) Abstract: The invention herein described, provides, among other things, self-buffering protein formulations. Particularly, the invention provides self-buffering pharmaceutical protein formulations that are suitable for veterinary and human medical use. The self-buffering protein formulations are substantially free of other buffering agents, stably maintain pH for the extended time periods involved in the distribution and storage of pharmaceutical proteins for veterinary and human medical use. The invention further provides methods for designing, making, and using the formulation. In addition to other advantages, the formulations avoid the disadvantages associated with the buffering agents conventionally used in current formulations of proteins for pharmaceutical use. The invention in these and other respects can be productively applied to a wide variety of proteins and is particularly useful for making and using self-buffering formulations of pharmaceutical proteins for veterinary and medical use, especially, in particular, for the treatment of diseases in human subjects.

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SELF-BUFFERING PROTEIN FORMULATIONS

Reference to Related Applications

This application is a continuation-in-part of and claims full priority benefit of U.S. Provisional Application Serial No. 60/690,582 filed 14 June 2005, which is incorporated herein by reference in its entirety.

Field of the Invention

The invention relates to the formulation of proteins, especially pharmaceutical proteins. In particular, it relates to self-buffering biopharmaceutical protein compositions, and to methods for designing, making, and using the compositions. It further relates to pharmaceutical protein compositions for veterinary and/or for human medical use, and to methods relating thereto.

Background of the Invention

Many aspects of pharmaceutical production and formulation processes are pH sensitive. Maintaining the correct pH of a finished pharmaceutical product is critical to its stability, effectiveness, and shelf life, and pH is an important consideration in designing formulations for administration that will be acceptable, as well as safe and effective.

To maintain pH, pharmaceutical processes and formulations use one or more buffering agents. A variety of buffering agents are available for pharmaceutical use. The buffer or buffers for a given application must be effective at the desired pH. They must also provide sufficient buffer capacity to maintain the desired pH for as long as necessary. A good buffer for a pharmaceutical composition must satisfy numerous other requirements as well. It must be appropriately soluble. It must not form deleterious complexes with metal ions, be toxic, or unduly penetrate, solubilize, or absorb on membranes or other surfaces. It should not interact with other components of the composition in any manner which decreases their availability or effectiveness. It must be stable and effective at maintaining pH over the range of conditions to which it will be exposed during formulation and during storage of the product. It must not be deleteriously affected by oxidation or other reactions occurring in its environment, such as those that occur in the processing of the composition in which it is providing the buffering action. If carried over or incorporated into a final product, a buffering agent

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must be safe for administration, compatible with other components of the composition over the shelf-life of the product, and acceptable for administration to the end user.

Although there are many buffers in general use, only a limited number are suitable for biological applications and, of these, fewer still are acceptable for pharmaceutical processes and formulations. As a result, it often is challenging to find a buffer that not only will be effective at maintaining pH but also will meet all the other requirements for a given pharmaceutical process, formulation, or product.

The challenge of finding a suitable buffer for pharmaceutical use can be especially acute for pharmaceutical proteins. The conformation and activity of proteins are critically dependent upon pH. Proteins are susceptible to a variety of pH sensitive reactions that are deleterious to their efficacy, typically many more than affect small molecule drugs. For instance, to mention just a few salient examples, the side chain amides of asparagine and glutamine are deamidated at low pH (less than 4.0) and also at neutral or high pH (greater than 6.0). Aspartic acid residues promote the hydrolysis of adjacent peptide bonds at low pH. The stability and disposition of disulfide bonds is highly dependent on pH, particularly in the presence of thiols. Solubility, flocculation, aggregation, precipitation, and fibrillation of proteins are critically dependent on pH. The crystal habit important to some pharmaceutical formulations also is critically dependent on pH. And pH is also an important factor in surface adsorption of many pharmaceutical peptides and proteins.

Buffering agents that catalyze reactions that inactivate and/or degrade one or more other ingredients, moreover, cannot be used in pharmaceutical formulations. Buffers for pharmaceutical use must have not only the buffer capacity required to maintain correct pH, but also they must not buffer so strongly that their administration deleteriously perturbs a subject's physiological pH. Buffers for pharmaceutical formulations also must be compatible with typically complex formulation processes. For instance, buffers that sublime or evaporate, such as acetate and imidazole, generally cannot be relied upon to maintain pH during lyophilization and in the reconstituted lyophilization product. Other buffers that crystallize out of the protein amorphous phase, such as sodium phosphate, cannot be relied upon to maintain pH in processes that require freezing.

Buffers used to maintain pH in pharmaceutical end-products also must be not only effective at maintaining pH but also safe and acceptable for administration to the subject. For instance, several otherwise useful buffers, such as citrate at low or high

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concentration and acetate at high concentration, are undesirably painful when administered parenterally.

Some buffers have been found to be useful in the formulation of pharmaceutical proteins, such as acetate, succinate, citrate, histidine (imidazole), phosphate, and Tris. They all have undesirable limitations and disadvantages. And they all have the inherent disadvantage of being an additional ingredient in the formulation, which complicates the formulation process, poses a risk of deleteriously affecting other ingredients, stability, shelf-life, and acceptability to the end user.

There is a need, therefore, for additional and improved methods of maintaining pH in the production and formulation of pharmaceuticals and in pharmaceutical compositions, particularly in the production and formulation of biopharmaceutical proteins and in biopharmaceutical protein compositions.

Summary

Therefore, it is among the various objects and aspects of the invention to provide, in certain of the preferred embodiments, protein formulations comprising a protein, particularly pharmaceutically acceptable formulations comprising a pharmaceutical protein, that are buffered by the protein itself, that do not require additional buffering agents to maintain a desired pH, and in which the protein is substantially the only buffering agent (i.e., other ingredients, if any, do not act substantially as buffering agents in the formulation).

In this regard and others, it is among the various objects and aspects of the invention to provide, in certain preferred embodiments, self-buffering formulations of a protein, particularly of a pharmaceutical protein, characterized in that the concentration of the formulated protein provides a desired buffer capacity.

It is further among the various objects and aspects of the invention to provide, in certain of the particularly preferred embodiments, self-buffering protein formulations, particularly pharmaceutical protein formulations, in which the total salt concentration is less than 150 mM..

It is further among the various objects and aspects of the invention to provide, in certain of the particularly preferred embodiments, self-buffering protein formulations, particularly pharmaceutical protein formulations, that further comprise one or more polyols and/or one or more surfactants.

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It is also further among the various objects and aspects of the invention to provide, in certain of the particularly preferred embodiments, self-buffering formulations comprising a protein, particularly a pharmaceutical protein, in which the total salt concentration is less than 150 mM, that further comprise one or more excipients, including but not limited to, pharmaceutically acceptable salts; osmotic balancing agents (tonicity agents); surfactants, polyols, anti-oxidants; antibiotics; antimycotics; bulking agents; lyoprotectants; anti-foaming agents; chelating agents; preservatives; colorants; and analgesics.

It is additionally among the various objects and aspects of the invention to provide, in certain preferred embodiments, self-buffering protein formulations, particularly pharmaceutical protein formulations, that comprise, in addition to the protein, one or more other pharmaceutically active agents.

Various additional aspects and embodiments of the invention are illustratively described in the following numbered paragraphs. The invention is described by way of reference to each of the items set forth in the paragraphs, individually and/or taken together in any combination. Applicant specifically reserves the right to assert claims based on any such combination.

1. A composition according to any of the following, wherein the composition has been approved for pharmaceutical use by a national or international authority empowered by law to grant such approval preferably the European Agency for the Evaluation of Medical Products, Japan's Ministry of Health, Labor and Welfare, China's State Drug Administration, United States Food and Drug Administration, or their successor(s) in this authority, particularly preferably the United States Food and Drug Administration or its successor(s) in this authority.

2. A composition according to any of the foregoing or the following, wherein the composition is produced in accordance with good manufacturing practices applicable to the production of pharmaceuticals for use in humans.

3. A composition according to any of the foregoing or the following, comprising a protein, the protein having a buffer capacity per unit volume per pH unit of at least that of approximately: 2.0 or 3.0 or 4.0 or 5.0 or 6.50 or 8.00 or 10.0 or 15.0 or 20.0 or 30.0 or 40.0 or 50.0 or 75.0 or 100 or 125 or 150 or 200 or 250 or 300 or 350 or 400 or 500 mM sodium acetate buffer in pure water over the range of pH 5.0 to 4.0 or pH 5.0 to 5.5, preferably as determined in accordance with the methods described in Example 1 and 2, particularly preferably at least 2.0 mM, especially particularly

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preferably at least 3.0 mM, very especially particularly preferably at least 4.0 mM or at least 5.0 mM, especially particularly preferably at least 7.5 mM, particularly preferably at least 10 mM, preferably at least 20 mM.

4. A composition according to any of the foregoing or the following wherein, exclusive of the buffer capacity of the protein, the buffer capacity per unit volume per pH unit of the composition is equal to or less than that of 1.0 or 1.5 or 2.0 or 3.0 or 4.0 or 5.0 mM sodium acetate buffer in pure water over the range of pH 4.0 to 5.0 or pH 5.0 to 5.5, preferably as determined in accordance with the methods described in Example 1 and 2, particularly preferably less than that of 1.0 mM, very especially particularly preferably less than that of 2.0 mM, especially particularly preferably less than that of 2.5 mM, particularly preferably less than that of 3.0 mM, preferably less than that of 5.0 mM.

5. A composition according to any of the foregoing or the following comprising a protein wherein over the range of plus or minus 1 pH unit from the pH of the composition, the buffer capacity of the protein is at least approximately: 1.00 or 1.50 or 1.63 or 2.00 or 3.00 or 4.00 or 5.00 or 6.50 or 8.00 or 10.0 or 15.0 or 20.0 or 30.0 or 40.0 or 50.0 or 75.0 or 100 or 125 or 150 or 200 or 250 or 300 or 350 or 400 or 500 or 700 or 1,000 mEq per liter per pH unit, preferably at least approximately 1.00, particularly preferably 1.50, especially particularly preferably 1.63, very especially particularly preferably 2.00, very highly especially particularly preferably 3.00, very especially particularly preferably 5.0, especially particularly preferably 10.0, particularly preferably 20.0.

6. A composition according to any of the foregoing or the following comprising a protein wherein over the range of plus or minus 1 pH unit from the pH of the composition, exclusive of the protein, the buffer capacity per unit volume per pH unit of the composition is equal to or less than that of 0.50 or 1.00 or 1.50 or 2.00 or 3.00 or 4.00 or 5.00 or 6.50 or 8.00 or 10.0 or 20.0 or 25.0 mM sodium acetate buffer in pure water over the range pH 5.0 to 4.0 or pH 5.0 to 5.5, particularly preferably determined in accordance with Example 1 and/or Example 2.

7. A composition according to any of the foregoing or the following, wherein over a range of plus or minus 1 pH unit from a desired pH, the protein provides at least approximately 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 99.5% of the buffer capacity of the composition, preferably at least approximately 75%, particularly preferably at least approximately 85%, especially

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particularly preferably at least approximately 90%, very especially particularly preferably at least approximately 95%, very highly especially particularly preferably at least approximately 99% of the buffer capacity of the composition.

8. A composition according to any of the foregoing or the following, wherein the concentration of the protein is between approximately: 20 and 400, or 20 and 300, or 20 and 250, or 20 and 200, or 20 and 150 mg/ml, preferably between approximately 20 and 400 mg/ml, particularly preferably between approximately 20 and 250, especially particularly between approximately 20 and 150 mg/ml.

9. A composition according to any of the foregoing or the following, wherein the pH maintained by the buffering action of the protein is between approximately: 3.5 and 8.0, or 4.0 and 6.0, or 4.0 and 5.5, or 4.0 and 5.0, preferably between approximately 3.5 and 8.0, especially particularly preferably approximately 4.0 and 5.5.

10. A composition according to any of the foregoing or the following, wherein the salt concentration is less than: 150 mM or 125 mM or 100 mM or 75 mM or 50 mM or 25 mM, preferably 150 mM, particularly preferably 125 mM, especially preferably 100 mM, very particularly preferably 75 mM, particularly preferably 50 mM, preferably 25 mM.

11. A composition according to any of the foregoing or the following, further comprising one or more pharmaceutically acceptable salts; polyols; surfactants; osmotic balancing agents; tonicity agents; anti-oxidants; antibiotics; antimycotics; bulking agents; lyoprotectants; anti-foaming agents; chelating agents; preservatives; colorants; analgesics; or additional pharmaceutical agents.

12. A composition according to any of the foregoing or the following, comprising one or more pharmaceutically acceptable polyols in an amount that is hypotonic, isotonic, or hypertonic, preferably approximately isotonic, particularly preferably isotonic, especially preferably any one or more of sorbitol, mannitol, sucrose, trehalose, or glycerol, particularly especially preferably approximately 5% sorbitol, 5% mannitol, 9% sucrose, 9% trehalose, or 2.5% glycerol, very especially in this regard 5% sorbitol, 5% mannitol, 9% sucrose, 9% trehalose, or 2.5% glycerol.

13. A composition according to any of the foregoing or the following, further comprising a surfactant, preferably one or more of polysorbate 20, polysorbate 80, other fatty acid esters of sorbitan, polyethoxylates, and poloxamer 188, particularly preferably polysorbate 20 or polysorbate 80, preferably approximately 0.001 to 0.1%

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polysorbate 20 or polysorbate 80, very preferably approximately 0.002 to 0.02% polysorbate 20 or polysorbate 80, especially 0.002 to 0.02% polysorbate 20 or polysorbate 80.

14. A composition according to any of the foregoing or the following, wherein the protein is a pharmaceutical agent and the composition is a sterile formulation thereof suitable for treatment of a non-human or a human subject.

15. A composition according to any of the foregoing or the following, wherein the protein is a pharmaceutical agent effective to treat a disease and the composition is a sterile formulation thereof suitable for administration to a subject for treatment thereof.

16. A composition according to any of the foregoing or the following, wherein the protein does not induce a significantly deleterious antigenic response following administration to a subject.

17. A composition according to any of the foregoing or the following, wherein the protein does not induce a significantly deleterious immune response following administration to a subject.

18. A composition according to any of the foregoing or the following, wherein the protein is a human protein.

19. A composition according to any of the foregoing or the following, wherein the protein is a humanized protein.

20. A method according to any of the foregoing or the following, wherein the protein is an antibody, preferably an IgA, IgD, IgE, IgG, or IgM antibody, particularly preferably an IgG antibody, very particularly preferably an IgG1, IgG2, IgG3, or IgG4 antibody, especially an IgG2 antibody.

21. A composition according to any of the foregoing or the following, wherein the protein comprises a: Fab fragment, Fab_2 fragment, Fab_3 fragment, Fc fragment, scFv fragment, bis-scFv(s) fragment, minibody, diabody, triabody, tetrabody, VhH domain, V-NAR domain, V_H domain, V_L domain, camel Ig, Ig NAR, or peptibody, or a variant, derivative, or modification of any of the foregoing.

22. A composition according to any of the foregoing or the following, wherein the protein comprises an Fc fragment or a part thereof or a derivative or variant of an Fc fragment or part thereof.

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23. A composition according to any of the foregoing or the following, wherein the protein comprises a first binding moiety of a pair of cognate binding moieties, wherein the first moiety binds the second moiety specifically.

24. A composition according to any of the foregoing or the following, wherein the protein comprises (a) an Fc fragment or a part thereof or a derivative or variant of an Fc fragment or part thereof, and (b) a first binding moiety of a pair of cognate binding moieties.

25. A composition according to any of claims 1, 5, 7, 9, 11, 13, or 14, wherein the protein is selected from the group consisting of proteins that bind specifically to one or more CD proteins, HER receptor family proteins, cell adhesion molecules, growth factors, nerve growth factors, fibroblast growth factors, transforming growth factors (TGF), insulin-like growth factors, osteoinductive factors, insulins and insulin-related proteins, coagulation and coagulation-related proteins, colony stimulating factors (CSFs), other blood and serum proteins blood group antigens; receptors, receptor-associated proteins, growth hormone receptors, T-cell receptors; neurotrophic factors. neurotrophins, relaxins, interferons, interleukins, viral antigens, lipoproteins, integrins, rheumatoid factors, immunotoxins, surface membrane proteins, transport proteins, homing receptors, addressins, regulatory proteins, and immunoadhesins,

26. A composition according to any of the foregoing or the following, wherein the protein is selected from the group consisting of: OPGL specific binding proteins, myostatin specific binding proteins, IL-4 receptor specific binding proteins, IL1-R1 specific binding proteins, Ang2 specific binding proteins, NGF-specific binding proteins, CD22 specific binding proteins, IGF-1 receptor specific binding proteins, B7RP-1 specific binding proteins, IFN gamma specific binding proteins, TALL-1 specific binding proteins, stem cell factors, Flt-3 ligands, and IL-17 receptors.

27. A composition according to any of the foregoing or the following, wherein the protein is selected from the group consisting of proteins that bind specifically to one ormore of: CD3, CD4, CD8, CD19, CD20, CD34; HER2, HER3, HER4, the EGF receptor; LFA-1, Mol, p150,95, VLA-4, ICAM-1, VCAM, alpha v/beta 3 integrin; vascular endothelial growth factor ("VEGF"); growth hormone, thyroid stimulating hormone, follicle stimulating hormone, luteinizing hormone, growth hormone releasing factor, parathyroid hormone, mullerian-inhibiting substance, human macrophage inflammatory protein (MIP-1-alpha), erythropoietin (EPO), NGF-

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beta, platelet-derived growth factor (PDGF), aFGF, bFGF, epidermal growth factor (EGF), TGF-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta4, TGF-beta5, IGF-I, IGF-II, des(1-3)-IGF-I (brain IGF-I), insulin, insulin A-chain, insulin B-chain, proinsulin, insulin-like growth factor binding proteins;, such as, among others, factor VIII, tissue factor, von Willebrands factor, protein C, alpha-1–antitrypsin, plasminogen activators, such as urokinase and tissue plasminogen activator ("t-PA"), bombazine, thrombin, and thrombopoietin; M-CSF, GM-CSF, G-CSF, albumin, IgE, flk2/flt3 receptor, obesity (OB) receptor, bone-derived neurotrophic factor (BDNF), NT-3, NT-4, NT-5, NT-6); relaxin A-chain, relaxin B-chain, prorelaxin; interferon-alpha, -beta, and -gamma;IL-1 to IL-10; AIDS envelope viral antigen; calcitonin, glucagon, atrial natriuretic factor, lung surfactant, tumor necrosis factor-alpha and -beta, enkephalinase, RANTES, mouse gonadotropin-associated peptide, Dnase, inhibin, and activin; protein A or D, bone morphogenetic protein (BMP), superoxide dismutase, decay accelerating factor (DAF).

28. A composition according to any of the foregoing or the following, wherein the protein is selected from the group consisting of:Actimmune (Interferon-gamma-1b), Activase (Alteplase), Aldurazme (Laronidase), Amevive (Alefacept), Avonex (Interferon beta-1a), BeneFIX (Nonacog alfa), Beromun (Tasonermin), Beatseron (Interferon-beta-1b), BEXXAR (Tositumomab), Tev-Tropin (Somatropin), Bioclate or RECOMBINATE (Recombinant), CEREZME (Imiglucerase), ENBREL (Etanercept), Eprex (epoetin alpha), EPOGEN/Procit (Epoetin alfa), FABRAZYME (Agalsidase beta), Fasturtec/Elitek ELITEK (Rasburicase), FORTEO (Teriparatide), GENOTROPIN (Somatropin), GlucaGen (Glucagon), Glucagon (Glucagon, rDNA origin), GONAL-F (follitropin alfa), KOGENATE FS (Octocog alfa), HERCEPTIN (Trastuzumab), HUMATROPE (SOMATROPIN), HUMIRA (Adalimumab), Insulin in Solution, INFERGEN® (Interferon alfacon-1), KINERET® (anakinra), Kogenate FS (Antihemophilic Factor), LEUKIN (SARGRAMOSTIM Recombinant human granulocyte-macrophage colony stimulating factor (rhuGM-CSF)), CAMPATH (Alemtuzumab), RITUXAN® (Rituximab), TNKase (Tenecteplase), MYLOTARG (gemtuzumab ozogamicin), NATRECOR (nesiritide), ARANESP (darbepoetin alfa), NEULASTA (pegfilgrastim), NEUMEGA (oprelvekin), NEUPOGEN (Filgrastim), NORDITROPIN CARTRIDGES (Somatropin), NOVOSEVEN (Eptacog alfa), NUTROPIN AQ (somatropin), Oncaspar (pegaspargase), ONTAK (denileukin diftitox), ORTHOCLONE OKT (muromonab-CD3), OVIDREL (choriogonadotropin

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alfa), PEGASYS (peginterferon alfa-2a), PROLEUKIN (Aldesleukin), PULMOZYME (dornase alfa), Retavase (Reteplase), REBETRON Combination Therapy containing REBETOL® (Ribavirin) and INTRON® A (Interferon alfa-2b), REBIF (interferon beta-1a), REFACTO (Antihemophilic Factor), REFLUDAN (lepirudin), REMICADE (infliximab), REOPRO (abciximab)ROFERON®-A (Interferon alfa-2a), SIMULECT (baasiliximab), SOMAVERT (Pegivisomant), SYNAGIS® (palivizumab), Stemben (Ancestim, Stem cell factor), THYROGEN, INTRON® A (Interferon alfa-2b), PEG-INTRON® (Peginterferon alfa-2b), XIGRIS® (Drotrecogin alfa activated), XOLAIR® (Omalizumab), ZENAPAX® (daclizumab), and ZEVALIN® (Ibritumomab Tiuxetan).

29. A composition according to any of the foregoing or the following, wherein the protein is Ab-hCD22 or a fragment thereof, or a variant, derivative, or modification of Ab-hCD22 or of a fragment thereof; Ab-hIL4R or a fragment thereof, or a variant, derivative, or modification of Ab-hIL4R or of a fragment thereof; Ab-hOPGL or a fragment thereof, or a variant, derivative, or modification of Ab-hOPGL or of a fragment thereof, or Ab-hB7RP1 or a fragment thereof, or a variant, derivative, or modification of Ab-hB7RP1 or of a fragment thereof.

30. A composition according to any of the foregoing or the following, wherein the protein is: Ab-hCD22 or Ab-hIL4R or Ab-hOPGL or Ab-hB7RP1.

31. A composition according to any of the foregoing or the following comprising a protein and a solvent, the protein having a buffer capacity per unit volume per pH unit of at least that of 4.0 mM sodium acetate in water over the range of pH 4.0 to 5.0 or pH 5.0 to 5.5, particularly as determined by the methods described in Examples 1 and 2, wherein the buffer capacity per unit volume of the composition exclusive of the protein is equal to or less than that of 2.0 mM sodium acetate in water over the same ranges preferably determined in the same way.

32. A composition according to any of the foregoing or the following comprising a protein and a solvent, wherein at the pH of the composition the buffer capacity of the protein is at least 1.63 mEq per liter for a pH change of the composition of plus or minus 1 pH unit wherein the buffer capacity of the composition exclusive of the protein is equal to or less than 0.81 mEq per liter at the pH of the composition for a pH change of plus or minus 1 pH unit.

33. A lyophilate which upon reconstitution provides a composition in accordance with any of the foregoing or the following.

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34. A kit comprising in one or more containers a composition or a lyophilate in accordance with any of the foregoing or the following, and instructions regarding use thereof.

35. A process for preparing a composition or a lyophilate according to any of the foregoing or the following, comprising removing residual buffer using a counter ion.

36. A process for preparing a composition or a lyophilate according to any of the foregoing or the following, comprising removing residual buffer using any one or more of the following in the presence of a counter ion: chromatography, dialysis, and/or tangential flow filtration.

37. A process for preparing a composition or a lyophilate according to any of the foregoing or the following, comprising removing residual buffer using tangential flow filtration.

38. A process for preparing a composition or a lyophilate according to any of the foregoing or the following comprising a step of dialysis against a solution at a pH below that of the preparation , and, if necessary, adjusting the pH thereafter by addition of dilute acid or dilute base.

39. A method for treating a subject comprising administering to a subject in an amount and by a route effective for treatment a composition according to any of the foregoing or the following, including a reconstituted lyophilate.

Brief Description of the Figures

Figure 1 depicts titration data and buffer capacity as a function of concentration for sodium acetate standard buffers over the range from pH 5.0 to 4.0. Panel A is a graph that depicts the pH change upon acid titration of several different concentrations of a standard sodium acetate buffer, as described in Example 1. pH is indicated on the vertical axis. The amount of acid added to each solution is indicated on the horizontal axis in microequivalents of HCl added per ml of solution (μEq/ml). The linear least squares trend lines are depicted for each dataset. Acetate concentrations are indicated in the inset. Panel B is a graph that depicts the buffer capacity of the acetate buffers over the acidic pH range as determined from the titration data depicted in Panel A, as described in Example 1. Buffer capacity is indicated on the vertical axis as microequivlents of acid per ml of buffer solution per unit change in pH (μEq/ml-pH). Acetate concentration is indicated on the horizontal axis in mM.

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Figure 2 depicts titration data and buffer capacity as a function of concentrations for sodium acetate standard buffers over the range from pH 5.0 to 5.5. Panel A is a graph that depicts the pH change upon base titration of several different concentration of a standard sodium acetate buffer, as described in Example 2. pH is indicated on the vertical axis. The amount of base added to each solution is indicated on the horizontal axis in microequivalents of NaOH added per ml of solution (μEq/ml). The linear least squares trend lines are depicted for each dataset. Acetate concentrations are indicated in the inset. Panel B is a graph that depicts the buffer capacity of the acetate buffers over the basic pH range as determined from the titration data depicted in Panel A and described in Example 2. Buffer capacity is indicated on the vertical axis as microequivlents of base per ml of buffer solution per unit change in pH (μEq/ml-pH). Acetate concentration is indicated on the horizontal axis in mM.

Figure 3 depicts the determination of acetate concentration in acetate buffer standards, as described in Example 3. The graph shows a standard curve for the determinations, with peak area indicated on the vertical axis and the acetate concentration indicated on the horizontal axis. The nominal and the measured amounts of acetate in the solutions used for the empirical determination of buffer capacity are tabulated below the graph.

Figure 4 is a graph that depicts the pH change upon acid titration of several different concentrations of Ab-hOPGL over the range of pH 5.0 to 4.0, as described in Example 4. pH is indicated on the vertical axis. The amount of acid added to the solutions is indicated on the horizontal axis in microequivalents of HCl added per ml of buffer solution (μEq/ml). The linear least squares trend lines are depicted for each dataset. Ab-hOPGL concentrations are indicated in the inset.

Figure 5 is a graph that depicts the pH change upon base titration of several different concentrations of Ab-hOPGL over the range 5.0 to 6.0, as described in Example 5. pH is indicated on the vertical axis. The amount of base added to the solutions is indicated on the horizontal axis in microequivalents of NaOH added per ml of buffer solution (μEq/ml). The linear least squares trend lines are depicted for each dataset. Ab-hOPGL concentrations are indicated in the inset.

Figure 6 shows the residual acetate levels in Ab-hOPGL solutions used for determining buffer capacity. The graph shows the standard curve used for the acetate determinations as described in Example 6. The nominal and the experimentally measured acetate concentrations in the solutions are tabulated below the graph.

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Figure 7 is a graph depicting the buffer capacity of Ab-hOPGL plus or minus residual acetate in the pH range 5.0 to 4.0. The data were obtained as described in Example 7. The upper line shows Ab-hOPGL buffer capacity with residual acetate. The lower line shows Ab-hOPGL buffer capacity adjusted for residual acetate. The vertical axis indicates buffer capacity in microequivalents of acid per ml of Ab-hOPGL solution per unit of pH (μEq/ml-pH). The horizontal axis indicates the concentration of Ab-hOPGL in mg/ml. The buffer capacities of different concentrations of standard acetate buffers as described in Example 1 are shown as horizontal lines. The concentrations of the buffers are indicated above the lines.

Figure 8 is a graph depicting the buffer capacity of Ab-hOPGL plus or minus residual acetate in the basic pH range pH 5.0 to 6.0. The data were obtained as described in Example 8. The upper line depicts Ab-hOPGL buffer capacity with residual acetate. The lower line depicts Ab-hOPGL buffer capacity adjusted for residual acetate. The vertical axis indicates buffer capacity in microequivalents of base added per ml of buffer solution per unit of pH (μEq/ml-pH). The horizontal axis indicates the concentration of Ab-hOPGL in mg/ml. The buffer capacities of several concentrations of standard sodium acetate buffers as described in Example 2 are indicated by horizontal lines. The acetate concentrations are indicated above each line.

Figure 9 depicts, in a pair of charts, pH and Ab-hOPGL stability in self-buffering and conventionally buffered formulations. Panel A depicts the stability of self-buffered Ab-hOPGL, Ab-hOPGL formulated in acetate buffer, and Ab-hOPGL formulated in glutamate as a function of storage time at 4° C over a period of six months. The vertical axis indicates Ab-hOPGL stability in percent Ab-hOPGL monomer determined by SE-HPLC. Storage time is indicated on the horizontal axis. Panel B depicts the pH of the same three formulations measured over the same period of time. The determinations of protein stability and the measurements of pH are described in Example 9.

Figure 10 depicts titration curves and buffer capacities for several concentrations of self-buffering Ab-hB7RP1 formulations over the range of pH 5.0 to 4.0. Panel A shows the titration data. pH is indicated on the vertical axis. The amount of acid added to the solutions is indicated on the horizontal axis in microequivalents of HCl added per ml of buffer solution (μEq/ml). The linear least squares trend lines are depicted for each dataset. The Ab-hB7RP1 concentrations are indicated in the inset. Panel B depicts the buffer capacities of Ab-hB7RP1 formulations. The upper line

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shows the buffer capacities for the formulations including the contribution of residual acetate. The lower line shows the buffer capacities for formulations after subtracting the contribution of residual acetate based on SE-HPLC determinations as described in Example 3. Linear least squares trend lines are shown for the two data sets. The vertical axis indicates buffer capacity in microequivalents of acid per ml of buffer solution per unit of pH (μEq/ml-pH). The concentration of Ab-hB7RP1 is indicated on the horizontal axis in mg/ml. The buffer capacities of several concentrations of standard sodium acetate buffers as described in Example 1 are shown by dashed horizontal lines. The acetate buffer concentration are shown below each line. The results were obtained as described in Example 10.

Figure 11 depicts titration curves and buffer capacities for several concentrations of self-buffering Ab-hB7RP1 formulations over the range of pH 5.0 to 6.0. Panel A shows the titration data. pH is indicated on the vertical axis. The amount of base added to the solutions is indicated on the horizontal axis in microequivalents of NaOH added per ml of buffer solution (μEq/ml). The linear least squares trend lines are depicted for each dataset. The Ab-hB7RP1 concentrations are indicated in the inset. Panel B depicts the buffer capacities of Ab-hB7RP1 formulations. The upper line shows the buffer capacities for the formulations containing residual acetate. The lower line shows the buffer capacities for formulations adjusted to remove the contribution of residual acetate. Linear least squares trend lines are shown for the two data sets. The vertical axis indicates buffer capacity in microequivalents of base per ml of buffer solution per unit of pH (μEq/ml-pH). The concentration of Ab-hB7RP1 is indicated on the horizontal axis in mg/ml. The buffer capacities of several concentrations of standard sodium acetate buffers as described in Example 2 are shown by dashed horizontal lines. The acetate buffer concentrations are shown above each line. The results were obtained as described in Example 11.

Figure 12 depicts Ab-hB7RP1 stability in self-buffering and conventionally buffered formulations at 4° C and 29° C. Panel A depicts the stability of self-buffered Ab-hB7RP1, Ab-hB7RP1 formulated in acetate buffer, and Ab-hB7RP1 formulated in glutamate as a function of storage at 4° C over a period of six months. The vertical axis depicts Ab-hB7RP1 monomer in the samples determined by SE-HPLC. Time is indicated on the horizontal axis. Panel B depicts the stability of the same three formulations as a function of storage at 29° C over the same period of time. Axes in

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Panel B are the same as in Panel A. The determinations of protein stability by HPLC-SE are described in Example 12.

Figure 13 depicts pH stability in self buffer formulations of Ab-hB7RP1 at 4° C and 29° C. The vertical axis indicates pH. Time, in weeks, is indicated on the horizontal axis. Temperatures of the datasets are indicated in the inset. The data were obtained as described in Example 13.

Figure 14 depicts the buffer capacity of self-buffering formulations of Ab-hCD22 as a function of Ab-hCD22 concentration over the range of pH 4.0 to 6.0. Panel A depicts the buffer capacities of self-buffering Ab-hCD22 formulations as a function of Ab-hCD22 concentration over the range of pH 4.0 to 5.0. Panel B depicts the buffer capacities of self-buffering Ab-hCD22 formulations as a function of concentration over the range of pH 5.0 to 6.0. In both panels the vertical axis indicates buffer capacity in microequivalents of base per ml of buffer solution per unit of pH (μEq/ml-pH), and the horizontal axis indicates Ab-hCD22 concentrations in mg/ml. For reference, the buffer capacity of 10 mM sodium acetate as described in Example 1 is shown in both panels by a dashed horizontal line. The results shown in the Figure were obtained as described in Example 14.

Figure 15 depicts titration curves and buffer capacities for several concentrations of self-buffering Ab-hIL4R formulations over the range of pH 5.0 to 4.0. Panel A shows the titration data. pH is indicated on the vertical axis. The amount of acid added to the solutions is indicated on the horizontal axis in microequivalents of HCl added per ml of buffer solution (μEq/ml). The linear least squares trend lines are depicted for each dataset. The Ab-hIL4R concentrations are indicated in the inset. Panel B depicts the buffer capacities of Ab-hIL4R as a function of concentration. The linear least squares trend line is shown for the dataset. The vertical axis indicates buffer capacity in microequivalents of base per ml of buffer solution per unit of pH (μEq/ml-pH). The concentration of Ab-hIL4R is indicated on the horizontal axis in mg/ml. The buffer capacities of several concentrations of standard sodium acetate buffers as described in Example 1 are shown by dashed horizontal lines. The acetate buffer concentrations are shown above each line. The results were obtained as described in Example 15.

Figure 16 depicts titration curves and buffer capacities for several concentrations of self-buffering Ab-hIL4R formulations over the range of pH 5.0 to 6.0. Panel A shows the titration data. pH is indicated on the vertical axis. The amount

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of base added to the solutions is indicated on the horizontal axis in microequivalents of NaOH added per ml of buffer solution (μEq/ml). The linear least squares trend lines are depicted for each dataset. The Ab-hIL4R concentrations are indicated in the inset. Panel B depicts the buffer capacities of Ab-hIL4R as a function of concentration. The linear least squares trend line is shown for the dataset. The vertical axis indicates buffer capacity in microequivalents of base per ml of buffer solution per unit of pH (μEq/ml-pH). The concentration of Ab-hIL4R is indicated on the horizontal axis in mg/ml. The buffer capacities of several concentrations of standard sodium acetate buffers as described in Example 2 are shown by dashed horizontal lines. The acetate buffer concentrations are shown above each line. The results were obtained as described in Example 16.

Figure 17 depicts Ab-hIL4R and pH stability in acetate buffered and self-buffered formulations of Ab-hIL4R at 37° C as a function of time. Panel A is a bar graph showing Ab-hIL4R stability over four weeks at 37° C. The vertical axis indicates stability in per cent monomeric Ab-hIL4R as determined by SE-HPLC. The horizontal axis indicates storage time in weeks. The insert identifies the data for the acetate and for the self-buffered formulations. Panel B shows the pH stability of the same formulations for the same conditions and time periods. The pH is indicated on the vertical axis. Storage time in weeks is indicated on the horizontal axis. Data for the acetate and self-buffered formulations are indicated in the inset. The data were obtained as described in Example 17.

Glossary

The meanings ascribed to various terms and phrases as used herein are illustratively explained below.

"A" or "an" herein means "at least one;" "one or more than one."

"About," unless otherwise stated explicitly herein, means ∀ 20%. For instance about 100 herein means 80 to 120, about 5 means 4 to 6, about 0.3 means 0.24 to 0.36, and about 60% means 48% to 72% (not 40% to 80%).

"Agonist(s)" means herein a molecular entity that is different from a corresponding stimulatory ligand but has the same stimulatory effect. For instance (although agonists work through other mechanisms), for a hormone that stimulates an

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activity by binding to a corresponding hormone receptor, an agonist is a chemically different entity that binds the hormone receptor and stimulates its activity.

"Antagonist(s)" means herein a molecular entity that is different from a corresponding ligand and has an opposite effect. For instance (although antagonists work through other mechanisms), one type of antagonist of a hormone that stimulates an activity by binding to a corresponding hormone receptor is a chemical entity that is different from the hormone and binds the hormone receptor but does not stimulate the activity engendered by hormone binding, and by this action inhibits the effector activity of the hormone.

"Antibody(s)" is used herein in accordance with its ordinary meaning in the biochemical and biotechnological arts.

Among antibodies within the meaning of the term as it is used herein, are those isolated from biological sources, including monoclonal and polyclonal antibodies, antibodies made by recombinant DNA techniques (also referred to at times herein as recombinant antibodies), including those made by processes that involve activating an endogenous gene and those that involve expression of an exogenous expression construct, including antibodies made in cell culture and those made in transgenic plants and animals, and antibodies made by methods involving chemical synthesis, including peptide synthesis and semi-synthesis. Also within the scope of the term as it is used herein, except as otherwise explicitly set forth, are chimeric antibodies and hybrid antibodies, among others.

The prototypical antibody is a tetrameric glycoprotein comprised of two identical light chain–heavy chain dimers joined together by disulfide bonds. There are two types of vertebrate light chains, kappa and lambda. Each light chain is comprised of a constant region and a variable region. The two light chains are distinguished by constant region sequences. There are five types of vertebrate heavy chains: alpha, delta, epsilon, gamma, and mu. Each heavy chain is comprised of a variable region and three constant regions. The five heavy chain types define five classes of vertebrate antibodies (isotypes): IgA, IgD, IgE, IgG, and IgM. Each isotype is made up of, respectively, (a) two alpha, delta, epsilon, gamma, or mu heavy chains, and (b) two kappa or two lambda light chains. The heavy chains in each class associate with both types of light chains; but, the two light chains in a given molecule are both kappa or both lambda. IgD, IgE, and IgG generally occur as "free" heterotetrameric glycoproteins. IgA and IgM generally occur in complexes comprising several IgA or

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several IgM heterotetramers associated with a "J" chain polypeptide. Some vertebrate isotypes are classified into subclasses, distinguished from one another by differences in constant region sequences. There are four human IgG subclasses, IgG1, IgG2, IgG3, and IgG4, and two IgA subclasses, IgA1 and IgA2, for example. All of these and others not specifically described above are included in the meaning of the term "antibody(s)" as used herein.

The term "antibody(s)" further includes amino acid sequence variants of any of the foregoing as described further elsewhere herein.

"Antibody-derived" as used herein means any protein produced from an antibody, and any protein of a design based on an antibody. The term includes in its meaning proteins produced using all or part of an antibody, those comprising all or part of an antibody, and those designed in whole or in part on the basis of all or part of an antibody. "Antibody-derived" proteins include, but are not limited to, Fc, Fab, and Fab_2 fragments and proteins comprising the same, V_H domain and V_L domain fragments and proteins comprising the same, other proteins that comprise a variable and/or a constant region of an antibody, in whole or in part, scFv(s) intrabodies, maxibodies, minibodies, diabodies, amino acid sequence variants of the foregoing, and a variety of other such molecules, including but not limited to others described elsewhere herein.

"Antibody-related" as used herein means any protein or mimetic resembling in its structure, function, or design an antibody or any part of an antibody. Among "antibody-related" proteins as the term is used herein are "antibody-derived" proteins as described above. It is to be noted that the terms "antibody-derived" and "antibody-related" substantially overlap; both terms apply to many such proteins. Examples of "antibody-related" proteins, without implying limitation in this respect, are peptibodies and receptibodies. Other examples of "antibody-related" proteins are described elsewhere herein.

"Antibody polypeptide(s)" as used herein, except as otherwise noted, means a polypeptide that is part of an antibody, such as a light chain polypeptide, a heavy chain polypeptide and a J chain polypeptide, to mention a few examples, including among others fragments, derivatives, and variants thereof, and related polypeptides.

"Approximately" unless otherwise noted means the same as about.

"Binding moiety(s)" means a part of a molecule or a complex of molecules that binds specifically to part of another molecule or complex of molecules. The binding

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moiety may be the same or different from the part of the molecule or complex of molecules to which it binds. The binding moiety may be all of a molecule or complex of molecules as well.

"Binds specifically" is used herein in accordance with its ordinary meaning in the art and means, except as otherwise noted, that binding is stronger with certain specific moieties than it is to other moieties in general, that it is stronger than non-specific binding that may occur with a wide variety of moieties, and that binding is selective for certain moieties and does not occur to as strong an extent with others. In the extreme case of specific binding, very strong binding occurs with a single type of moiety, and there is no non-specific binding with any other moiety.

"Co-administer" means an administration of two or more agents in conjunction with one another, including simultaneous and/or sequential administration.

"Cognate(s)" herein means complementary, fitting together, matching, such as, for instance, two jigsaw puzzles that fit one another, the cylinder mechanism of a lock and the key that opens it, the substrate binding site of an enzyme and the substrate of the enzyme, and a target and target binding protein that binds specifically thereto.

"Cognate binding moieties" herein means binding moieties that bind specifically to one another. Typically, but not always, it means a pair of binding moieties that bind specifically to one another. The moieties responsible for highly selective binding of a specific ligand and ligand receptor provide an illustrative example of cognate binding moieties. Another example is provided by the moieties that binds an antigen and an antibody.

"Composition" means any composition of matter comprising one or more constituents, such as a formulation.

"Comprised of" is a synonym of "comprising" (see below).

"Comprising" means including, without further qualification, limitation, or exclusion as to what else may or may not be included. For example, "a composition comprising x and y" means any composition that contains x and y, no matter what else it may contain. Likewise, "a method comprising x" is any method in which x is carried out, no matter what else may occur.

"Concentration" is used herein in accordance with its well-known meaning in the art to mean the amount of an item in a given amount of a mixture containing the item, typically expressed as a ratio. For example, concentration of a solute, such as a protein in a solution, can be expressed in many ways, such as (but not limited to): (A)

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Weight Percent (i) = weight of solute per 100 units of solvent volume; (B) Weight Percent (ii) = weight of solute per 100 units of total weight; (C) Weight Percent (iii) = weight of solute per 100 units of solvent by weight; (D) Mass Percent = mass of solute per 100 mass units of solution; (E) Mole Fraction = moles of solute per total moles of all components; (F) Molarity = moles of solute per liter of solution (*i.e.*, solute plus solvent); (G) Molality = moles of solute per Kg of solvent; and (H) Volume Molality = moles of solute per liter of solvent.

"Control region(s)" is used herein in accordance with its well-known meaning in the art, and except as noted otherwise, refers to regions in DNA or proteins that are responsible for controlling one or more functions or activities thereof. For instance, "expression control region" with reference to the control of gene expression, means the regions in DNA that are required for transcription to occur properly and that are involved in regulating when transcription occurs, how efficiently it occurs, when it is stopped, and the like.

"*De novo*" is used herein in accordance with its well-known meaning in the art, to denote something made from new. For instance, a *de novo* amino acid sequence is one not derived from a naturally occurring amino acid sequence, although, such a *de novo* sequence may have similarities with a naturally occurring sequence. *De novo* amino acid sequences can be generated, for instance, by *a priori* design, by combinatorial methods, by selection methods. They can be made, for example, by chemical synthesis, by semi-synthesis, and by a variety of recombinant DNA techniques, all of which are well know to those skilled in the art.

"Deleterious" means, as used herein, harmful. By way of illustration, "deleterious" processes include, for example, harmful effects of disease processes and harmful side effects of treatments.

"Derivative(s) " is used herein to mean derived from, in substance, form, or design, such as, for instance, a polypeptide that is based on but differs from a reference polypeptide, for instance, by alterations to its amino acid sequence, by fusion to another polypeptide, or by covalent modification.

"Disease(s)" a pathology, a condition that deleteriously affects health of a subject.

"Disorder(s)" a malediction, a condition that deleteriously alters health.

"Dysfunction" means, as used herein, a disorder, disease, or deleterious effect of an otherwise normal process.

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"Effective amount" generally means an amount which provides the desired local or systemic effect. For example, an effective amount is an amount sufficient to effectuate a beneficial or desired clinical result. The effective amount can be provided all at once in a single administration or in fractional amounts that provide the effective amount in several administrations. The precise determination of what would be considered an effective amount may be based on factors individual to each subject, including their size, age, injury, and/or disease or injury being treated, and amount of time since the injury occurred or the disease began. One skilled in the art will be able to determine the effective amount for a given subject based on these considerations which are routine in the art. As used herein, "effective dose" means the same as "effective amount."

"Effective route" generally means a route which provides for delivery of an agent to a desired compartment, system, or location. For example, an effective route is one through which an agent can be administered to provide at the desired site of action an amount of the agent sufficient to effectuate a beneficial or desired clinical result.

"Endogenous" (such as endogenous gene) is used herein to refer to, for instance, genes and other aspects of DNA, such as control regions, that naturally occur in a genome and organism, unless otherwise indicated.

"Exogenous" (such as exogenous gene), unless otherwise indicated, is used herein generally to mean, for instance, DNA from an outside source, such as DNA introduced to a cell and incorporated into its genome.

"FBS" means fetal bovine serum.

"Formulation(s)" means a combination of at least one active ingredient with one or more other ingredients for one or more particular uses, such as storage, further processing, sale, and/or administration to a subject, such as, for example, administration to a subject of a specific agent in a specific amount, by a specific route, to treat a specific disease.

"Fragment(s)" herein means part of a larger entity, such as a part of a protein; for instance, a polypeptide consisting of less than the entire amino acid sequence of a larger polypeptide. As used herein, the term includes fragments formed by terminal deletion and fragments formed by internal deletion, including those in which two or more non-contiguous portions of a polypeptide are joined together to form a smaller polypeptide, which is a fragment of the original.

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"Fusion protein(s)" herein means a protein formed by fusing all or part of two polypeptides, which may be either the same or different. Typical fusion proteins are made by recombinant DNA techniques, by end to end joining of nucleotides encoding the two (or more) polypeptides.

"Genetically engineered" herein means produced using a deliberate process of genetic alteration, such as by recombinant DNA technology, classical methods of genetic manipulation, chemical methods, a combination of all three, or other methods.

"Homolog(s)" herein means having homology to another entity, such as a protein that is homologous to another protein. Homologous means resembling in structure or in function.

"Ionization" herein means the change of net charge on a substance by at least one, including loss or gain of charge, such as the ionization of acetic acid in low pH solution, from HOAc to OAc^- and H^+.

"k" herein denotes an equilibrium co-efficient, in accordance with its standard meaning in chemistry.

"k_a" herein denotes the dissociation constant of a particular hydrogen of a molecule, in accordance with its standard meaning in chemistry, such as, for example, the dissociation constant of the acidic hydrogen of acetic acid.

"k_d" herein denotes a dissociation constant of a pair of chemical entities (or moieties), in accordance with its standard meaning in chemistry.

"Kit" means a collection of items used together for a given purpose or purposes.

"Ligand(s)" herein means a molecular entity that binds selectively and stoichiometrically to one or more specific sites on one more other molecular entities. Binding typically is non-covalent, but can be covalent as well. A very few examples, among many others, are (a) antigens, which typically bind non-covalently to the binding sites on cognate antibodies; (b) hormones, which typically bind hormone receptors, non-covalently; (c) lectins, which bind specific sugars, non-covalently; (d) biotins, which bind multiple sites on avidin and other avidin-like proteins, non-covalently; (e) hormone antagonists, which bind hormone receptors and inhibit their activity and/or that of the corresponding hormone; and (f) hormone agonists, which similarly bind hormone receptors but stimulate their activity.

"Ligand-binding moiety(s)" herein means a molecular entity that binds a ligand, typically, a part of a larger molecular entity that binds the ligand, or a molecular entity derived therefrom.

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"Ligand-binding protein(s)" herein means a protein that binds a ligand.

"Ligand moiety(s)" herein means a molecular entity that binds to a ligand-binding molecular entity in much the same way as does the corresponding ligand. A ligand moiety can be all of a ligand, or part of it, derived from a ligand, or generated *de novo*. Typically, however, the ligand moiety is more or less exclusively the aspect thereof that binds corresponding ligand-binding entities. The ligand moiety need not comprise, and the term generally does not denote, structural features other than those required for ligand binding.

"mEq" herein means milliequivalent(s).

"μEq" herein means microequivalent(s).

"Mimetic(s)" herein means a chemical entity with structural or functional characteristics of another, generally unrelated chemical entity. For instance, one kind of hormone mimetic is a non-peptide organic molecule that binds to the corresponding receptor in the same way as the corresponding hormone.

"mM" means millimolar; 10^{-3} moles per liter.

"Modified protein(s)," "modified polypeptide(s)," or "modified fragment(s)" herein means a protein or a polypeptide or a fragment of a protein or polypeptide comprising a chemical moiety (structure) other than those of the twenty naturally occurring amino acids that form naturally occurring proteins. Modifications most often are covalently attached, but can also be attached non-covalently to a protein or other polypeptide, such as a fragment of a protein.

"Moiety(s)" herein means a molecular entity that embodies a specific structure and/or function, without extraneous components. For instance, in most cases, only a small part of a ligand-binding protein is responsible for ligand binding. This part of the protein, whether continuously encoded or discontinuously, is an example of a ligand-binding moiety.

"Naturally occurring" means occurs in nature, without human intervention.

"Non-naturally occurring" means does not occur in nature or, if it occurs in nature, is not in its naturally occurring state, environment, circumstances, or the like.

"PBS" means phosphate buffered saline.

"Peptibody" refers to a molecule comprising an antibody Fc domain (i.e., C_H2 and C_H3 antibody domains) that excludes antibody C_H1, C_L, V_H, and V_L domains as well as Fab and F(ab)2, wherein the Fc domain is attached to one or more peptides, preferably a pharmacologically active peptide, particularly preferably a randomly

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generated pharmacologically active peptide. The production of peptibodies is generally described in PCT publication WO00/24782, published May 4, 2000, which is herein incorporated by reference in its entirety, particularly as to the structure, synthesis, properties, and uses of peptibodies.

"Peptide(s)" herein means the same as polypeptide; often, but not necessarily, it is used in reference to a relatively short polypeptide,

"pH" is used in accordance with its well-known and universal definition as follows:

$pH = - \log [H_3O^+]$.

"Pharmaceutical" as used herein means is acceptable for use in a human or non-human subject for the treatment thereof, particularly for use in humans, and approved therefor by a regulatory authority empowered to regulate the use thereof such as , for example, the Food and Drug Administration in the United States, European Agency for the Evaluation of Medicinal Products, Japan's Ministry of Health, Labor and Welfare, or other regulatory agency such as those listed in R. Ng, DRUGS: FROM DISCOVERY TO APPROVAL, Wiley-Liss (Hoboken, NJ) (2004), which is herein incorporated by reference in its entirety, particularly as to regulatory authorities concerned with drug approval, especially as listed in Chapter 7. As used herein the phrase "wherein the composition has been approved for pharmaceutical use by an authority legally empowered to grant such approval" means an entity or institution or the like, established by law and by law charged with the responsibility and power to regulate and approve the use of drugs for use in humans, and in some cases, in non-humans. Approval by any one such agency anywhere meets this qualification. It is not necessary for the approving agency to be that of the state in witch, for instance, infringement is occurring. Example of such entities include the U. S Food and Drug Administration and the other agencies listed herein above.

As used herein, "pharmaceutical" also may refer to a product produced in accordance with good manufacturing practices, such as those described in, among others, Chapter 9 and Chapter 10, of R. Ng, DRUGS: FROM DISCOVERY TO APPROVAL, Wiley-Liss (Hoboken, NJ) (2004), which is herein incorporated by reference in its entirety, particularly in parts pertinent to good manufacturing practices for pharmaceutical protein formulations, in particular, as set forth in Chapters 9 and 10.

"Pharmaceutically acceptable" is used herein in accordance with its well-known meaning in the art to denote that which is acceptable for medical or veterinary use,

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preferably for medical use in humans, particularly approved for such use by the US Food and Drug Administration or other authority as described above regarding the meaning of "pharmaceutical."

"Polypeptide(s)" see "Protein(s)."

"Precursor(s)" is used herein in accordance with its well-known meaning in the art to denote an entity from which another entity is derived. For instance, a precursor protein is a protein that undergoes processing, such as proteolytic cleavage or modification, thereby giving rise to another precursor protein (which will undergo further processing) or a mature protein.

"Protein(s)" herein means a polypeptide or a complex of polypeptides, in accordance with its well-known meaning in the art. As used herein, "protein(s)" includes both straight chain and branched polypeptides. It includes unmodified and modified polypeptides, including naturally occurring modifications and those that do not occur naturally. Such modifications include chemical modifications of the termini, the peptide backbone, and the amino acid side chains; amino acid substitutions, deletions and additions; and incorporation of unusual amino acids and other moieties, to name just a few such modifications. It also includes "engineered" polypeptides and complexes thereof, such as, but not limited to, any polypeptide or complex of polypeptides that has been deliberatively altered in its structure by, for instance, recombinant DNA techniques, chemical synthesis, and/or covalent modification, including deliberate alteration of amino acid sequence and/or posttranslational modifications.

"Protonation" means the addition of at least one hydrogen.

"Self-buffering" means the capacity of a substance, such as a pharmaceutical protein, to resist change in pH sufficient for a given application, in the absence of other buffers.

"Semi-*de novo*" herein means (a) partly designed in accordance with a particular reference and or produced from a precursor, and (b) partly designed without reference to a particular reference (such as designed solely by general principles and not based on any particular reference). For example, a polypeptide made by producing a first peptide in a bacterial expression system, producing a second peptide by chemical synthesis, and then joining the two peptides together to form the polypeptide.

"Semi-synthesis" means as used herein a combination of chemical and non-chemical methods of synthesis.

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"Subject" means a vertebrate, such as a mammal, such as a human. Mammals include, but are not limited to, humans, farm animals, sport animals, and pets. Subjects in need of treatment by methods and/or compositions of the present invention include those suffering from a disorder, dysfunction, or disease, or a side effect thereof, or from a side effect of a treatment thereof.

"Substantially" is used herein in accordance with its plain and ordinary definition to mean to a great extent or degree. For example, substantially complete means complete to a great extent, complete to a great degree. By way of further illustration, substantially free of residue means to a great extent free of residue, free of residue to a great degree. Should numerical accuracy be required, depending on context, "substantially," as used herein means, at least, 80% or more, particularly 90% or more, very particularly 95% or more.

"Therapeutically effective" is used herein in accordance with its well-known meaning in the art to denote that which achieves an improvement in the prognosis or condition of a subject or that otherwise achieves a therapeutic objective, including, for instance, a reduction in the rate of progress of a disease even if a subject's condition, nonetheless, continues to deteriorate.

"Therapeutically effective amount" generally is used to qualify the amount of an agent to encompass those amounts that achieve an improvement in disorder severity. For example, effective neoplastic therapeutic agents prolong the survivability of the subject, inhibit the rapidly-proliferating cell growth associated with the neoplasm, or effect a regression of the neoplasm. Treatments that are therapeutically effective within the meaning of the term as used herein, include treatments that improve a subject's quality of life even if they do not improve the disease outcome per se.

"Treat," "treating," or "treatment" are used broadly in relation to the invention and each such term encompasses, among others, preventing, ameliorating, inhibiting, or curing a deficiency, dysfunction, disease, or other deleterious process, including those that interfere with and/or result from a therapy.

"Variant(s)" herein means a naturally occurring or synthetic version of, for instance, a protein that is structurally different from the original but related in structure and/or function, such as an allelic variant, a paralog, or a homolog of a protein.

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Description of the Invention

The invention provides for the first time self-buffering protein formulations, particularly biopharmaceutical protein formulations, methods for making the formulations, and methods for using the formulations, among other things. Any protein that provides sufficient buffer capacity within the required pH range at a concentration suitable for its intended use can be prepared as a self-buffering protein formulation in accordance with the invention. The invention can be practiced with a variety of proteins, including both naturally-occurring proteins and "engineered" proteins, particularly biopharmaceutical proteins, as discussed further below.

The utility of proteins, particularly biopharmaceutical proteins, to be formulated in self-buffering compositions, particularly pharmaceutically acceptable compositions, has not been recognized prior to the invention herein disclosed. The influence of proteins in the regulation of physiological pH has been recognized and studied for some time. However, it has not heretofore been recognized that proteins, particularly biopharmaceutical proteins, can have enough buffer capacity to maintain a formulation within a desired pH range, without additional buffering agents.

Biopharmaceutical proteins for use in the United States are formulated as buffered solutions, unbuffered solutions, amorphous or crystalline suspensions, and lyophilates.

Most of the buffered solution formulations use a conventional buffering agent. Two proteins, Pulmozyme® and Humulin®, are formulated as solutions without conventional buffering agents. Neither of these proteins provides substantial self-buffering capacity in the formulations.

Pulmozyme® has a molecular weight of about 37,000 Daltons and contains 5 histidines, 22 aspartic acids, and 12 glutamic acids, among its 260 amino acids. The buffering capacity of the protein within 0.5 pH units of pH 6.3 is determined substantially by its histidine content. On this basis, the upper limit of the self-buffering capacity of the formulation is determined by the effective concentration of the histidine residues, 0.15 mM. The molar concentration of aspartic acid and glutamic acid in the formation is 0.9 mM. The total molar concentration of all three amino acids together, thus, is just a little over 1 mM, at the concentration of the formulation.

Humulin® is formulated at 3.5 g/ml. It has a molecular weight of about 6,000 Daltons and contains 2 aspartic acids, 8 glutamic acids, and 2 histidines. None of these amino acids is a particularly effective buffer at the pH of the formulation: 7.0 to 7.8.

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At this concentration the molar concentration of histidines, which are closest in pK_a to the pH of the formulation, is 1.16 mM.

The biopharmaceutical lyophilates are reconstituted prior to use forming solutions or suspensions. Most of the lyophilates contain conventional buffers that maintain the proper pH of the reconstituted formulations. A few others, in which the protein concentration is low or the pH must be low (less than 3) or high (greater than 9.5), are, effectively unbuffered.

Thus, buffering is achieved in current biopharmaceutical protein formulations using conventional buffering agents. The ability of proteins by themselves to buffer pharmaceutical protein formulations has not been fully appreciated and has not been used for the manufacture of protein pharmaceuticals.

The determination of protein buffer capacity, typically, is important to developing self-buffering protein formulations in accordance with the invention. Pertinent thereto, methods for measuring buffer capacity and for determining the buffer capacity of proteins are described below. To allow ready comparability of data, protein buffer capacity must be expressed in comparable units and/or related to a buffer standard. Accordingly, the following section describes pH metrics and standards that meet these requirements, in accordance with the invention.

1. Buffering

A widely accepted definition of buffering is the resistance to change in pH of a composition upon addition of acid or base. Buffer capacity thus often is defined as the ability of a composition to resist pH change.

Typically buffer capacity is expressed in terms of the amount of strong acid or base required to change the pH of a composition a given amount. Van Slyke provided the most widely used quantitative measure of buffer capacity, according to which, for a solution, buffer capacity is expressed as the amount of strong acid or base required to change the pH of one liter of the solution by one pH unit under standard conditions of temperature and pressure.

According to this measure, for instance, the buffer capacity of 1 liter of 5 mM HOAc, 5 mM NaOAc, pH 4.76 in pure water is 4.09×10^{-3} moles of a univalent strong base (*i.e.*, 4.09×10^{-3} equivalents of base), which can be calculated as follows.

The Henderson-Hasselbalch equation for the solution is:

$$pH = \log \{[5\ mM]\ NaOAc / [5\ mM]\ HOAc\} + 4.76$$

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Accordingly, the concentration, X, of a univalent strong base required to increase the pH of this buffer is:

4.76 to 5.76 is 5.76 = log {[5 mM + X mM] NaOAc / [5 mM - X mM] HOAc} + 4.76

Thus:

1.00 = log {[5 mM + X mM] NaOAc / [5 mM - X mM] HOAc}

10.0 = [5 mM + X mM] NaOAc / [5 mM - X mM] HOAc

10.0 = (5 mM + X mM) / (5 mM - X mM)

50 mM - 10X mM= 5 mM + X mM

11X mM = 45 mM

X = 4.09 mM,

which, for one liter yields:

(4.09 x 10^{-3} moles / liter)(1 liter)(1 equivalent / mole) = 4.09 x 10^{-3} equivalents

Thus, according to this measure, the buffer capacity of 1 liter of a 10mM acetate buffer containing 5 mM NaOAc and 5 mM HOAC at a pH of 4.76 in pure water is 4.09 x 10^{-3} equivalents of base per liter per pH unit. Put other ways, the buffer capacity of the solution is 4.09 milliequivalents of base per liter per pH unit, 4.09 microequivalents of base per milliliter per pH unit, 0.409 microequivalents of base per 100 microliters per pH unit, 40.9 nanomoles of base per 10 microliters per pH unit, and 4.09 nanonmoles of base per microliter per pH unit.

The same calculation yields the following buffer capacity for other concentrations of this acetate buffer at pH 4.76. A 2 mM acetate buffer as above has a buffer capacity of 0.818 mEq per liter per pH unit. At 4 mM the buffer capacity is 1.636 mEq per liter per pH unit. The capacity at 5 mM is 2.045 mEq per liter per pH unit. At 7.5 mM the capacity is 3.068 mEq per liter per pH unit. At 10 mM the acetate buffer has a buffer capacity of 4.091 mEq per liter per pH unit. At 15 mM its capacity is 6.136 mEq per liter per pH unit.

It is worth noting that an acetate buffer solution at the pK_a of acetic acid (pH 4.76) is equimolar in acetic acid and acetate base. (i.e., at the pK_a the acid and base are present in equal amounts). As a result, the resistance to change in pH (buffer capacity) of an acetate buffer at the pK_a of acetic acid is the same for addition of acid and base. The equipoise to acid and base is a general characteristic of buffering agents in buffers at a pH equal to their pK_a.

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At any other pH a buffer will contain different amounts of acid and base forms and, therefore, its resistance to change (i.e., its buffer capacity) upon addition of acid will not be the same as its resistance to change upon addition of base. As a result, it is preferable to define the capacity of such buffers in terms of (i) the amount of acid required to lower the pH by one unit, and (ii) the amount of base required to raise the pH by one unit.

The partitioning in a buffer between acid and base forms in a given composition, such as a pH standard, can be calculated at any pH and buffer concentration using the procedures set forth above in describing the buffer capacity of 10 mM NaOAc at pH 4.76 plus or minus (containing equimolar amounts of acetic acid and sodium acetate). And the results can be used to define the buffer capacity of a standard for reference use.

Thus, for instance, the partitioning of acetic acid into acetic acid and acetate base in a solution at pH 5.0 can be calculated readily using the foregoing procedures, and from this the buffer capacity can be calculated for both base and for acid addition. Calculated this way, the theoretical buffer capacity of 10 mM sodium acetate buffer over the range from pH 5.0 to 5.5 is approximately 2.1 mM per 0.5 pH unit and 4.2 mM per pH unit. Put another way, the buffer capacity of the buffer, theoretically, is approximately 4.2 μEq per ml of buffer solution per unit of pH change. Similarly, the theoretical buffer capacity of 10 mM sodium acetate buffer over the range from pH 5.0 to 4.0 is 4.9 mM, and, put another way, 4.9 μEq per ml of buffer per unit of pH change over a given range of pH.

While such calculations often are quite useful in many cases, empirical standards and empirical determinations are preferred. Among particularly preferred empirical standards are sodium acetate buffers over the range of pH 5.0 to 4.0 and pH 5.0 to 5.5 as exemplified in Examples 1 and 2. Especially preferred are sodium acetate buffers in accordance therewith in which the total acetate concentration is, in particular, 10 mM, preferably 5 mM, especially 4 mM, among others as set forth elsewhere herein.

Acetate buffers at pH 5.0 are more resistant to change in pH upon addition of acid than upon addition of base, as discussed above. In a preferred empirical standard of buffer capacity, the buffer capacity of a standard acetate buffer such as these is defined as: (i) the slope of the least squares regression line calculated for base titration data for the buffer from pH 5.0 to pH 5.5, and (ii) the slope of the least squares regression line calculated for acid titration data for the buffer from pH 5.0 to pH 4.0.

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The preparation of standard acetate buffers and the determination of their buffer capacities are described in Examples 1, 2, and 3. It is to be appreciated that much the same methods can be used to establish and use buffer capacity standards using other suitable buffering agents.

In measuring the buffer capacity of a self-buffering protein composition in accordance with the invention, it often is convenient to express the buffer capacity in terms of the concentration of a standard buffer at the same pH having the same buffer capacity. When a standard is used that is not at the pK_a of the buffering agent, such as a sodium acetate buffer initially at pH 5.0, in accordance with the invention the self-buffering composition is defined as having a buffer capacity equal to or greater than that of the standard, if either its buffer capacity upon base titration or its buffer capacity upon acid titration (or both) is equal to or exceeds the corresponding buffer capacity of the standard.

It is to be further appreciated that the pH of self-buffering protein compositions in accordance with the invention generally will not be at the pK_a of the self-buffering protein, or any acid-base substituent therein. Indeed proteins are polyprotic and, as discussed herein, often will have several substituents, each with a somewhat different pK_a that contribute to its buffer capacity in a given pH range. Accordingly, the buffer capacity of self-buffering protein formulations in accordance with the invention preferably is determined empirically by both acid titration and base titration over a given range of pH change from the desired pH of the composition. In preferred embodiments in this regard, the buffer capacity is determined by titrating with acid and separately with base over a change of respectively + and - 1 pH unit from the starting pH of the formulation. In particularly preferred embodiments, the titration data is collected for a change in pH of plus or minus 0.5 pH units. As described in the Examples, the buffer capacity is the slope of the least squares regression line for the data for pH as a function of equivalents of acid or base added to the composition over the range of titration.

a. Empirical Measures and Standards of Buffer Capacity

In certain preferred embodiments of the invention, the measure of buffer capacity is an empirical standard. Among preferred empirical standards in this regard are a particular volume of an aqueous solution at a particular temperature and a particular pH, containing a particular buffering agent at a particular concentration and

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either no other components than water, or one or more other particular components, each at a particular concentration.

A particularly preferred specific standard for determining buffer capacity in accordance with various aspects and preferred embodiments of the invention is 10 mM sodium acetate pH 5.00 in pure water free of other constituents at 21°C in equilibrium with ambient air at 1 atmosphere, as described in Examples 1 and 2, preferably expressed in equivalents per unit volume per pH unit, such as μEq/ml-pH. Buffer capacity of the standard should be measured empirically as described in Examples 1, 2, and 3, and as further discussed elsewhere herein.

A particularly preferred specific standard for determining buffer capacity in accordance with various aspects and preferred embodiments of the invention is 10 mM sodium acetate pH 4.76 in pure water free of other constituents at 21°C in equilibrium with ambient air at 1 atmosphere, as described in Examples 1 and 2, preferably expressed in equivalents per unit volume per pH unit, such as μEq/ml-pH. Buffer capacity of the standard should be measured empirically as described in Examples 1, 2, and 3, and as further discussed elsewhere herein. According to the Henderson-Hasselbalch equation, as noted above, the calculated buffer capacity of this standard over the range of pH 4.76 plus or minus 1 pH unit is 4.09 microequivalents per milliliter per pH unit (4.09 μEq/ml-pH).

A variety of other buffers are available for use as standards in other ranges of pH in accordance with various aspects and preferred embodiments of the invention in this regard. Reference buffers are particularly preferred in this regard, such as those well-known and routinely employed for analytical chemistry determinations. A variety of such buffering agents are set forth in textbooks on analytical chemistry and in monographs on the accurate determination of pH and buffer capacity.

Also useful in the invention in this regard are biological buffers, such as those described in, among other texts: TEITZ TEXTBOOK OF CLINICAL CHEMISTRY, 3rd Ed., Burtis and Ashwood, eds., W.B. Saunders Company, Philadelphia, PA (1999), in particular in Tables 50-13 to 50-16, which are herein incorporated by reference in their entireties as to buffering agents and buffers and their use as pH and/or buffer capacity standards in accordance with the invention in this respect; THE TOOLS OF BIOCHEMISTRY, Terrance G. Cooper, John Wiley & Sons, New York, NY (1977), in particular Chapter 1, pages 1-35, which is herein incorporated by reference in its entirety as to buffering agents and buffers and their use as pH and buffer capacity

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standards in accordance with the invention in this respect, most particularly as to Tables 1-3, 1-4, and 1-5 and text relating thereto, and PROTEIN PURIFICATION PRINCIPLES AND PRACTICE, 3rd Ed., Robert K. Scopes, Springer-Verlag, New York, NY (1994), in particular pages 160-164, especially therein Tables 6.4 and 6.5 and text relating thereto, Chapter 12, section 3, pages 324-333, especially therein Tables 12-4 and 12-5 and text relating thereto, and all of Appendix C: Buffers for Use in Protein Chemistry, which are herein incorporated by reference in their entirety as to buffering agents and buffers and their use in accordance with the invention in this respect.

Since some dissolved gases in water react with OH^- and/or H_3O^+, however, the empirically determined buffer capacity of the standard solution may vary somewhat from the theoretical value. Hence, the definition of the standard requires that the solution be in equilibrium with the atmosphere at a pressure of 1 atmosphere. In addition, the buffer standard must be held in and contacted only with materials that do not alter its components or its buffer capacity, such as those that leach acids, bases, or other reactants that may alter the effective concentration or activity of the acetate buffer in any way that would alter its buffer capacity. Given both of the foregoing, atmospheric equilibration and inertness of the container, buffer capacity of the standard will scale directly and linearly with its volume. Accordingly, the buffer capacity of 100 ml will be 1/10 that of 1.00 liter, and the buffer capacity of 10 ml will be 1/100 that of 1.00 liter. Accordingly, the volume of the standard can be adjusted for convenience and then normalized back to 1 liter as desired.

It may not always be convenient to make the foregoing 10 mM acetate buffer capacity standard for field use. However, a variety of other buffer capacity standards can be made and used in the same way as the acetate buffer, using a variety of other buffering agents. Provided only that the buffering standards are prepared properly, they can be calibrated against the acetate buffering standard described above and then used in the field. The results obtained with such alternative standards may then be expressed in terms of the foregoing acetate standard without substantial distortion or error.

The buffer capacity of such alternative standards also can be calibrated by calculation. To do so, the buffer capacity of the alterative standard is determined directly and expressed in mEq per unit volume per unit of pH. Determinations based on the alternative standard then can be normalized to the acetate standard using the ratio between the buffering capacities expressed in mEq per unit volume per unit of pH of the alternative and the acetate standards.

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Using such methods, which are commonly employed in metrology to relate field standards back to a reference standard, the acetate buffer standard described above provides a portable, scalable, reliable, and accurate reference for determining the buffer capacity of any composition that readily can be compared with disparate measures made on other compositions using similar methods.

b. Preparation of Buffer Capacity Standards

Buffer capacity standards can be prepared using well-established methods of analytical chemistry. See for instance, ANALYTICAL CHEMISTRY, 3rd Ed., Douglas A. Skoog and Donald M. West, Holt, Rinehart and Winston, New York (1979), particularly chapter 9 (pages 186-226), chapter 10 (pages 227-233), and methods described on pages 583-588; TEITZ TEXTBOOK OF CLINICAL CHEMISTRY, 3rd Ed., Burtis and Ashwood, eds., W.B. Saunders Company, Philadelphia, PA (1999), in particular Chapter 1 regarding general laboratory techniques for preparing and calibrating buffers and Tables 50-13 to 50-16; THE TOOLS OF BIOCHEMISTRY, Terrance G. Cooper, John Wiley & Sons, New York, NY (1977), in particular Chapter 1, pages 1-35, and Tables 1-3, 1-4, and 1-5 and text relating thereto; PROTEIN PURIFICATION PRINCIPLES AND PRACTICE, 3rd Ed., Robert K. Scopes, Springer-Verlag, New York, NY (1994), in particular pages 160-164, especially therein Tables 6.4 and 6.5 and text relating thereto, Chapter 12, section 3, pages 324-333, especially therein Tables 12-4 and 12-5 and text relating thereto, and all of Appendix C: Buffers for Use in Protein Chemistry; and REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY, 21st Ed., Beringer *et al.* Editors, Lippincott, Williams & Wilkins, Philadelphia, PA (2005), particularly in parts relating to buffering agents, buffers, buffer capacity and the like; each of which is herein incorporated by reference in its entirety particularly as to the preparation and use of buffers and buffer capacity standards in accordance with the invention in this respect.

The water used for preparing buffer capacity standards should be highly purified, preferably Type I water, such as milliQ water, or triple distilled water. The buffer reagents should be pure and, in particular, free of any substance that can alter the pH or buffer capacity of the standard solution, such as Reference Grade or ACS Reagent Grade reagents suitable for use in demanding analytic chemical analyses, as described in the foregoing references, TEITZ and REMINGTON cited above in

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particular, which are hereby incorporated by reference in their entireties particularly in parts pertinent to analytical grade water and reagents.

The exact compositions of the buffer reagents must be well established. The molecular weight of the buffer reagents must be known accurately for each buffer reagent. The molecular weights must be for the exact reagent that will be used and must include the weight of adducts such as hydrates that are present in the reagent. The effective number of hydrogen donors or hydrogen acceptors per molecule must be known accurately for each buffer reagent. The proportional distribution of different forms, such as hydrates, must be known for each reagent that contains a mixture of such forms. Concentrations of liquid buffer reagents much be known exactly, preferably in moles/volume and in moles/mass (*e.g.*, moles/liter and moles/gm or kg. Hygroscopic agents must be dried to remove moisture so that reagent can be accurately weighed.

Generally speaking, the information provided by well-established vendors of reagents and reference grade chemicals is sufficiently accurate for the preparation of buffer capacity standards as described above. And well-known standard techniques routinely employed in analytical chemistry can be used to dry "hygroscopic reagents" so that they can be weighed accurately.

As described therein, well established and routinely employed analytical chemistry methods can be employed to prepare and calibrate acid and base solutions, such as 1 N HCl and 1 N NaOH (to name just two) for titrating buffer capacity standard solutions, as well as sample protein solutions, to determine buffer capacity. It should be noted that the preparation of NaOH solutions for titration should be done so as to eliminate inaccuracies that arise from the interaction of certain dissolved gases with basic solutions, and the pH altering effects of their solvation. See for instance Skoog and West (1979) and other references cited above regarding the preparation and calibration of buffers and buffer standards, which are herein incorporated by reference in their entireties particularly in parts pertinent to the preparation of standard solutions for titration, as discussed above.

c. Empirical Measurement of Buffer Capacity

Titration of standards and samples to determine buffer capacity can be done using well- known, routine methods. Titrations can be carried out manually. They also can be carried out using an autotitrator. A wide variety of autotitrators that are suitable

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for use in the invention in this regard are commercially available from numerous vendors. Methods suitable for use in the invention in this regard are the same as those described in the references cited above regarding preparation and calibration of buffer standards, each of which is incorporated herein by reference in its entirety particularly in parts pertinent to the titration of known and unknown solutions to determine their buffer capacity.

2. Buffering by Proteins and Protein Buffer Capacity

a. Determination of Protein Hydrogen Equilibria and Buffer Capacity

Proteins invariably contain many acidic and basic constituents. As a result hydrogen ion equilibrium of proteins is highly complex. In fact, a complete description of the hydrogen ion equilibria of a protein in a given environment is beyond the reach of current theoretical and computational methods. Empirical measurements of protein buffer capacities, thus are preferred. Methods developed for precise empirical measurement of protein hydrogen equilibria, which can be and are routinely employed by those skilled in the art, are well-suited to measuring the buffering properties of proteins pertinent to the development of self-buffering protein formulations in accordance with the invention. Thus, the pH titration curves of proteins can be determined in accordance with the invention by well-known methods such as those described in and exemplified by pH titration studies of Tanford and co-workers on ribonuclease. See C. Tanford, "Hydrogen Ion Titration Curves of Proteins," in T. Shedlovsky (ed.), ELECTROCHEMISTRY IN BIOLOGY AND MEDICINE, John Wiley and Sons, New York, 1955, Ch. 13; C. Tanford and J.D. Hauenstein, *J. Am. Chem. Soc.* 78, 5287 (1956), C. Tanford, PHYSICAL CHEMISTRY OF MACROMOLECULES, John Wiley and Sons, New York, 1961, particularly pages 554-567, all of which are herein incorporated by reference particularly in parts pertinent to hydrogen ion titration of proteins and to the determination of buffering action and buffer capacity of proteins.

However, the present invention does not require such precise determinations as those described in the foregoing references. Rather, the buffering properties and buffer capacity of proteins in accordance with the invention can be determined using the methods described in standard references on analytical chemistry and biochemistry, such as, for instance, Skoog (1979), Cooper (1977), and Scopes (1994), cited above, each of which is herein incorporated by reference in its entirety particularly as to the

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empirical determination of titration curves, particularly of proteins within a given range of pH in accordance with the invention.

The determination of titration curves and buffer capacity in accordance with the invention is described in detail for numerous acetate buffers and a variety of pharmaceutical proteins in the Examples below. Thus, the pH titration curves of proteins can be determined empirically in accordance with such methods as described in the foregoing references over particular limited ranges of pH that are of interest to a given formulation. In many respects these methods are the same as those used in analytical chemistry for the titration of small molecules such as acetate buffers (as illustrated in the Examples). Somewhat greater care must be taken, however, in handling proteins to maintain the conformation and function required for effective formulation.

Protein titrations may be carried out manually or using automated titrators. Equipment for manual titration and automated titrators are readily available from a large number of suppliers and vendors. Methods suitable for determining pH titration curves and buffer capacity of proteins are exemplified in the Examples by reference to titration of acetate buffer standards and to titration of several different therapeutic proteins over defined ranges of pH. These methods can be employed to determine the hydrogen ionization behavior and buffer capacity of any other protein in accordance with the invention.

It is a particular aspect of the invention to determine the buffer capacity of proteins as a function of concentration in solution. In a preferred method in this regard, solutions of a given protein are prepared in a graded series of concentrations. A pH titration curve is determined for the protein at each concentration over the pH range of interest. Preferably titration curves are determined for the range of interest using both base titration and acid titration. The data are, in certain preferred embodiments, plotted on a graph of equivalents of acid or base added versus the measured pH of each solution. Typically, for ranges of about 0.5 to 1.0 pH unit, the titration data for each concentration closely fit a straight line, preferably determined by a least squares regression analysis. In preferred embodiments in this regard, buffer capacity for the protein at each concentration is equated to the slope of the regression line, expressed in units of equivalents per ml per pH unit (or fractions thereof). Also useful in the invention in this regard is the relationship between the buffer capacity of the protein and its concentration. In certain preferred embodiments, this relationship is determined

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by a least squares regression analysis of the best straight line fit of the buffer capacity data determined in accordance with the foregoing plotted on a graph of buffer capacity versus protein concentration.

Empirical data on the buffer capacity of proteins in accordance with the invention preferably is related to the buffer capacity of a standard acetate buffer. That is, in particularly preferred embodiments of the invention in this regard, the buffer capacity of a given protein at a given concentration in a given formulation, determined as above, is equated to the concentration of a standard acetate buffer having the same buffer capacity.

While empirical determinations as described herein are generally a crucial aspect of formulating self-buffering compositions in accordance with various aspects and preferred embodiments of the invention, theoretical and computational methods also can be productively employed to guide the design, manufacture, and use of such compositions (in conjunction with empirical determinations), as described below.

b. Prediction of Protein Hydrogen Ion Equilibria and Buffer Capacity

The ionization of hydrogen in proteins is complex but can be broken down in general terms into pH ranges defined by the ionizable hydrogens of amino acid side chains, and the terminal amino and carboxyl groups. The pK_a of terminal carboxyls in polypeptides typically ranges around 3.1. The pK_a of the acidic hydrogens in the side chains of aspartic acid and glutamic acid range around 4.4. The pK_a of histidine in polypeptides ranges around 6.0. The terminal amino group hydrogen ionization pK_a typically ranges around 7.5. The sulfhydryl in cysteine has a pK_a range around 8.5. The tyrosine hydroxyl and the lysine amine both have pK_as ranging around 10. The pK_a of arginine ranges around 12.

Conformational folding typically partitions large polypeptides and proteins in polar solvents into exposed solvent-accessible regions and more or less non-polar core regions that have little or no contact with the ambient environment. Folding produces many environments between these two extremes. Furthermore, the micro environment around a given amino acid side chain in a protein typically is affected by one or more of: solvent effects; binding of ions; chelation; complexation; association with co-factors; and post-translational modifications; to name just a few possibilities. Each of these can influence the pK_a of a given amino acid ionization in a protein. The pK_as for

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specific residues in a given protein, thus, can vary dramatically from that of a free amino acid.

Indeed, the perturbation of pK_as by microenvironments of amino acids in proteins has been used to study the folding of proteins and the disposition and charge state of specific amino acids in folded proteins. The protein titration curves reported by Tanford and others are complex with a few broad features in common. Typically only some of the ionizable protons are accounted for in the titration curves. Others apparently are located in the core and are inaccessible to solvent. The pK_as of individual side chains of the same type that can be detected in some cases can be distinguished from one another. Nonetheless, while detectably different, their pK_as generally are close to that of the free amino acid.

The strongest buffering action of proteins does not generally occur at the isoelectric point, as may be mistakenly supposed. In fact, buffering depends on the amino acid side chain hydrogens and the terminal hydrogens, and therefore occurs in ranges spanning the pK_as of the ionizable hydrogens in the free amino acids, as discussed above. The most important of these, for formulating compositions of proteins, especially certain pharmaceutical proteins that are more soluble and/or more stable, among other things, at weakly acidic pH (pH 4 to 6), is buffering action that occurs in the range of the pK_as of the carboxyl hydrogen of the amino acids aspartic acid and glutamic acid; that is, pH 4.0 to 5.5, particularly around 4.5.

There are a variety of ways available for estimating the buffer capacity of a given protein in a given solution at a given pH. Methods range from highly technical and complex computer models to those that can be carried out on a hand calculator. None of the methods is complete or entirely accurate; but, they can in some instances provide useful estimates.

For instance, a potentially useful idea of buffer capacity in some instances may be calculated for a protein in a solution based on its amino acid composition, the pK_as (in the solvent in question) of the terminal amine and carboxy groups and the amino acid side hydrogen donors and acceptors, the concentration of the protein, and the pH of the solution.

For example, a potentially useful estimate of the buffer capacity of a protein at pH in the range of the pK_a of the side chain carboxyl hydrogen of glutamic acid (as a free amino acid), can be gained from the molecular weight of the protein and the number of glutamic acid residues it contains. Dividing the former by the latter provides

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the weight per equivalent of glutamic acid and, therefore, the weight per equivalent of ionizable hydrogen at the pK_a of glutamic acid. Since glutamic acid and aspartic acid side chain carboxyl groups have nearly the same pK_as, results of such calculations for the two should be added together to yield an estimate of buffer capacity in a range around both their pK_as. The estimated buffer capacity of a solution of the protein at the pK_a can be calculated from the protein's concentration in the solution and the intrinsic factor just provided, namely weight per equivalent of ionizable hydrogen. Dividing the concentration by the weight per equivalent yields an estimate for the buffer capacity in units of Eq/volume. Such estimates often will be too high, since some residues usually are sequestered in regions of the protein not accessible to the solvent, and, therefore, do not contribute to its actual buffer capacity. It may be possible in certain instances to account for the effect of sequestering on buffer capacity. For instance, a fractional coefficient that reflects theoretical or empirical estimates of sequestering can be applied to adjust the original calculation.

Such calculations generally will be of less utility and less accurate than empirical determinations of protein buffer capacity, in accordance with the methods described elsewhere herein. But they can be useful to provide rough maximum estimates of the buffer capacity of proteins in solution.

3. Proteins

The invention herein disclosed may be practiced with any protein that provides sufficient buffer capacity in a desired pH range within the parameters of protein concentration and the like required for a desired formulation. Among preferred proteins in this regard are pharmaceutical proteins for veterinary and/or human therapeutic use, particularly proteins for human therapeutic use. Also among preferred proteins are proteins that are soluble in aqueous solutions, particularly those that are soluble at relatively high concentrations and those that are stable for long periods of time. Additionally, among preferred proteins are those that have a relatively high number of solvent accessible amino acids with side chain hydrogen ionization constants near the pH of the desired buffering action.

Further among preferred proteins of the invention are proteins for pharmaceutical formulations that do not induce a highly deleterious antigenic response following administration to a subject. Preferred in this regard are proteins for

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veterinary and/or human medical use, particularly, regarding the latter, humanized and human proteins.

Further among preferred proteins of the invention are proteins that bind selectively to specific targets, including ligand-binding proteins and protein ligands. Antigen-binding proteins, proteins derived therefrom, and proteins related thereto are among the particularly preferred embodiments of the invention in this regard. Highly preferred proteins of the invention in this regard are antibodies and proteins derived from antibodies or incorporating antibodies, in whole or part, including, to name just a few such entities: monoclonal antibodies, polyclonal antibodies, genetically engineered antibodies, hybrid antibodies, bi-specific antibodies, single chain antibodies, genetically altered antibodies, including antibodies with one or more amino acid substitutions, additions, and/or deletions (antibody muteins), chimeric antibodies, antibody derivatives, antibody fragments, which may be from any of the foregoing and also may be similarly engineered or modified derivatives thereof, fusion proteins comprising an antibody or a moiety derived from an antibody or from an antibody fragment, which may be any of the foregoing or a modification or derivative thereof, conjugates comprising an antibody or a moiety derived from an antibody, including any of the foregoing, or modifications or derivatives thereof, and chemically modified antibodies, antibody fragments, antibody fusion proteins, and the like, including all of the foregoing.

a. Antibodies, Antibody-Derived, and Antibody-Related Proteins and the Like

Among particularly preferred proteins in accordance with the invention are antibody polypeptides, such as heavy and light chain polypeptides that have the same amino acid sequence as those that occur in and make up naturally-occurring antibodies, such as those that occur in sera and antisera, including such polypeptides and proteins isolated from natural sources, as well as those that are made by hybridoma technologies, by activation of an endogenous gene (by homologous or non-homologous recombination, for instance), by expression of an exogenous gene under the control of an endogenous transcription control region, by expression of an exogenous expression construct, by semi-synthesis and by *de novo* synthesis, to name some techniques commonly employed for making antibodies and antibody-related polypeptides and

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proteins that can be used to produce antibody polypeptides and proteins in accordance with the invention.

Included among these antibody-related polypeptides and proteins are those in whole or part having a *de novo* amino acid sequence, those that comprise all or one or more parts of an antibody (that is: a continuous chain of amino acids having the same sequence as any four or more residues in the amino acid sequence of a naturally occurring antibody polypeptide), those having an amino acid sequence that matches in some way that of a naturally occurring antibody, but differs from it in other ways, those that have the same but different amino acid sequences as a naturally occurring counterpart or sequence relating thereto, but differ from the counterpart in one or more post-translational modifications, and those comprised in part of any of the foregoing (in part or in whole) fused to one or more polypeptide regions that can be of or derived from or related to a second, different antibody polypeptide, and can be of or derived from any other polypeptide or protein, whether naturally occurring, resembling but differing therefrom, having a semi-*de novo* amino acid sequence and/or a *de novo* sequence, among others. Such hybrids are generally referred to herein as fusion polypeptides and/or fusion proteins.

Further among preferred proteins in accordance with the invention herein described are modified proteins in accordance with all of the foregoing. Included among such modified proteins are proteins modified chemically by a non-covalent bond, covalent bond, or both a covalent and non-covalent bond. Also included are all of the foregoing further comprising one or more post-translational modifications which may be made by cellular modification systems or modifications introduced *ex vivo* by enzymatic and/or chemical methods, or introduced in other ways.

Among preferred proteins of the invention in this regard are Fab fragment(s), such as those produced by cleaving a typical dimeric $(LH)_2$ antibody with certain protease that leave the light chain intact while cleaving the heavy chains between the variable region and the adjacent constant region, "above" the disulfide bonds that hold the heavy chains together. Such cleavage releases one Fc fragment comprising the remaining portions of the heavy chains linked together, and two dimeric Fab fragments each comprising an intact light chain and the variable region of the heavy chain. Fab fragments also can be produced by other techniques that do not require isolation of a naturally occurring antibody and/or cleavage with a protease.

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Also preferred are Fab_2 fragment(s) such as those produced in much the same manner as Fab fragments using a protease that cleaves "between or below" the disulfide bonds. As a result, the two Fab fragments are held together by disulfide bonds and released as a single Fab_2 fragment. Fab_2 fragments can be produced by many other techniques including those that do not require isolation of an intact antibody or cleavage with a protease having the required specificity. Furthermore, both mono- and bi-specific Fab_2 fragments can now be made by a variety of routine techniques.

Also among preferred proteins in this regard are Fab_3 fragments, which are engineered antibody fragments in which three Fab fragments are linked together. Fab_3 fragments can be mono-, bi-, or tri-specific. They can be made in a variety of ways well-known to those of skill in the pertinent arts.

Among other preferred proteins in this regard are Fc fragments(s), such as those produced by cleavage with a protease in the same manner used for the production of either Fab fragments or Fab_2 fragments. However, for the production of Fc fragments, the dimeric heavy chain containing fragments are isolated rather than the light chain containing fragments. Fc fragments lack antigen combining sites, but comprise effector regions that play a role in physiological processes involving antibodies. Fc fragments can be made by a variety of techniques that are well-known and routinely employed by those of skill in the art for this purpose.

Among other preferred proteins in this regard are single-chain variable fragments ("scFv(s)"). scFv(s) are fusion proteins made by joining the variable regions of the heavy and light chains of an immunoglobulin. The heavy and light chains in an scFv typically are joined by a short serine, glycine linker. scFv(s) have the same specificity as the antibodies from which they were derived. Originally produced through phage display, scFv(s) now can be made by a variety of well-known methods.

Also preferred are Bis-scFv(s) which are fusions of two scFv(s). Bis-scFv(s) can be mono- or bi-specific. A variety of methods are well-known and can be applied in making Bis-scFv(s) in accordance with the invention.

Also preferred in accordance with the invention in this regard are minibodies; mono- and bi-specific diabodies; mono-, bi-, and tri-specific triabodies; mono-, bi-, tri-, and tetra-specific tetrabodies; VhH domains; V-NAR domains; V_H domains; V_L domains; camel Igs; Ig NARs; and others.

Also among preferred embodiments in accordance with various aspects and preferred embodiments of the invention in these and other regards are proteins

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comprising one or more CDR and/or CDR-derived and/or CDR-related regions of an antibody or one or more FR and/or FR-derived and/or FR-related regions of an antibody. In this regard CDR means complementary determining region; that is, a hypervariable region of a light or heavy chain of an antibody, typically about 9 to 12 amino acids in length that usually is an important part of an antigen specific binding moiety of an antibody. FR in this regard means a framework region of an antibody; that is, a region of about 15 to 20 amino acids that separates CDRs in the antigen specific binding moiety of an antibody. The terms CDR-derived and CDR-related, and the terms FR-derived and FR-related have the same meanings as to CDR and FR, respectively, as set forth in the above Glossary for the terms antibody-derived and antibody-related as to the term antibody.

Regarding antibodies, antibody-derived, and antibody-related proteins in accordance with the foregoing and with other aspects of the invention herein disclosed, see, for instance, *Protein Engineering: Principles and Practice*, Jeffrey L. Cleland and Chares S. Craik, eds. Wiley-Liss, Inc., New York (1996), particularly therein Kelley, Robert F., "Engineering Therapeutic Antibodies," Chapter 15, pp. 399-434 and Hollinger, P. & Hudson, P., "Engineered antibody fragments and the rise of single domains," *Nature Biotechnology*, September 2005, 1126-1136, each of which is herein incorporated by reference in its entirety particularly in parts pertinent to the structure and engineering of antibodies, particularly biopharmaceutical antibodies, and antibody-derived and antibody-related proteins, particularly antibody-derived and antibody-related pharmaceutical proteins in accordance with the invention herein described.

As to all of the foregoing, particularly preferred in the invention are human, humanized, and other proteins that do not engender a significantly deleterious immune responses when administered to a human. Also preferred in the invention are proteins in accordance with all the foregoing that similarly do not cause a significantly deleterious immune responses on administration to non-humans.

Among very particularly preferred proteins in accordance with the invention in these regards are fusion proteins comprising antibodies and/or antibody-derived proteins, polypeptides, or fragments or the like, including all of those described above. Among very particularly preferred fusion proteins of the invention in this regard are fusion proteins comprising an antibody or antibody-derived protein or fragment such as those described above and a ligand-binding moiety, such as those illustratively described herein.

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b. Target Binding Proteins

Also among preferred proteins of the invention in this regard are antibodies and other types of target binding proteins, and proteins relating thereto or derived therefrom, and protein ligands, and proteins derived therefrom or relating thereto. Among especially preferred ligand-binding proteins in this regard are proteins that bind signal and effector proteins, and proteins relating thereto or derived therefrom.

Among such binding proteins, including antibodies, including proteins derived therefrom and proteins related thereto, are those that bind to one or more of the following, alone or in any combination:

(i) CD proteins including but not limited to CD3, CD4, CD8, CD19, CD20, and CD34;

(ii) HER receptor family proteins, including, for instance, HER2, HER3, HER4, and the EGF receptor;

(iii) cell adhesion molecules, for example, LFA-1, Mol, p150,95, VLA-4, ICAM-1, VCAM, and alpha v/beta 3 integrin;

(iv) growth factors, including but not limited to, for example, vascular endothelial growth factor ("VEGF"); growth hormone, thyroid stimulating hormone, follicle stimulating hormone, luteinizing hormone, growth hormone releasing factor, parathyroid hormone, mullerian-inhibiting substance, human macrophage inflammatory protein (MIP-1-alpha), erythropoietin (EPO), nerve growth factor, such as NGF-beta, platelet-derived growth factor (PDGF), fibroblast growth factors, including, for instance, aFGF and bFGF, epidermal growth factor (EGF), transforming growth factors (TGF), including, among others, TGF-alpha and TGF-beta, including TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta4, or TGF-beta5, insulin-like growth factors-I and -II (IGF-I and IGF-II), des(1-3)-IGF-I (brain IGF-I), and osteoinductive factors;

(v) insulins and insulin-related proteins, including but not limited to insulin, insulin A-chain, insulin B-chain, proinsulin, and insulin-like growth factor binding proteins;

(vi) coagulation and coagulation-related proteins, such as, among others, factor VIII, tissue factor, von Willebrands factor, protein C, alpha-1–antitrypsin, plasminogen activators, such as urokinase and tissue plasminogen activator ("t-PA"), bombazine, thrombin, and thrombopoietin;

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(vii) colony stimulating factors (CSFs), including the following, among others, M-CSF, GM-CSF, and G-CSF;

(viii) other blood and serum proteins, including but not limited to albumin, IgE, and blood group antigens;

(ix) receptors and receptor-associated proteins, including, for example, flk2/flt3 receptor, obesity (OB) receptor, growth hormone receptors, and T-cell receptors;

(x) neurotrophic factors, including but not limited to, bone-derived neurotrophic factor (BDNF) and neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6);

(xi) relaxin A-chain, relaxin B-chain, and prorelaxin;

(xii) interferons, including for example, interferon-alpha, -beta, and -gamma;

(xiii) interleukins (ILs), e.g., IL-1 to IL-10;

(xiv) viral antigens, including but not limited to, an AIDS envelope viral antigen;

(xv) lipoproteins, calcitonin, glucagon, atrial natriuretic factor, lung surfactant, tumor necrosis factor-alpha and -beta, enkephalinase, RANTES (regulated on activation normally T-cell expressed and secreted), mouse gonadotropin-associated peptide, Dnase, inhibin, and activin;

(xvi) integrin, protein A or D, rheumatoid factors, immunotoxins, bone morphogenetic protein (BMP), superoxide dismutase, surface membrane proteins, decay accelerating factor (DAF), AIDS envelope, transport proteins, homing receptors, addressins, regulatory proteins, immunoadhesins, antibodies; and

(xvii) biologically active fragments or variants of any of the foregoing.

As to all of the foregoing, particularly preferred are those that are effective therapeutic agents, particularly those that exert a therapeutic effect by binding a target, particularly a target among those listed above, including targets derived therefrom, targets related thereto, and modifications thereof.

c. Particular Illustrative Proteins

Among particular illustrative proteins are certain antibody and antibody-related proteins, including peptibodies, such as, for instance, those listed immediately below and elsewhere herein:

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OPGL specific antibodies and peptibodies and the like (also referred to as RANKL specific antibodies, peptibodies and the like), including fully humanized and human OPGL specific antibodies, particularly fully humanized monoclonal antibodies, including but not limited to the antibodies described in International Publication Number WO 03/002713, which is incorporated herein in its entirety as to OPGL specific antibodies and antibody related proteins, particularly those having the sequences set forth therein, particularly, but not limited to, those denoted therein: 9H7; 18B2; 2D8; 2E11; 16E1; and 22B3, including the OPGL specific antibodies having either the light chain of SEQ ID NO: 2 as set forth therein in Figure 2 and/or the heavy chain of SEQ ID NO:4, as set forth therein in Figure 4, each of which is individually and specifically incorporated by reference herein in its entirety fully as disclosed in the foregoing publication. Acid and base titrations of an OPGL specific antibody ("Ab-hOPGL") over the pH ranges of 4.5 to 5.0 and 5.0 to 5.5 are described in the Examples below. The calculation of buffer capacity of Ab-hOPGL in these pH ranges also is described in the Examples below.

Myostatin binding agents or peptibodies, including myostatin specific peptibodies, particularly those described in US Application Publication Number 2004/0181033, which is incorporated by reference herein in its entirely particularly in parts pertinent to myostatin specific peptibodies, including but not limited to peptibodies of the mTN8-19 family, including those of SEQ ID NOS: 305-351, including TN8-19-1 through TN8-19-40, TN8-19 con1 and TN8-19 con2; peptibodies of the mL2 family of SEQ ID NOS: 357-383; the mL15 family of SEQ ID NOS: 384-409; the mL17 family of SEQ ID NOS: 410-438; the mL20 family of SEQ ID NOS: 439-446; the mL21 family of SEQ ID NOS: 447-452; the mL24 famuly of SEQ ID NOS: 453-454; and those of SEQ ID NOS: 615-631, each of which is individually and specifically incorporated by reference herein in its entirety fully as disclosed in the foregoing publication.

IL-4 receptor specific antibodies, particularly those that inhibit activities mediated by binding of IL-4 and/or IL-13 to the receptor, including those described in International Publication No. WO 2005/047331 of International Application Number PCT/US2004/03742, which is incorporated herein by reference in its entirety particularly in parts pertinent to IL-4 receptor specific antibodies, particularly such antibodies as are described therein, particularly, and without limitation, those designated therein: L1H1; L1H2; L1H3; L1H4; L1H5; L1H6; L1H7; L1H8; L1H9;

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L1H10; L1H11; L2H1; L2H2; L2H3; L2H4; L2H5; L2H6; L2H7; L2H8; L2H9; L2H10; L2H11; L2H12; L213; L2H14; L3H1; L4H1; L5H1; L6H1, each of which is individually and specifically incorporated by reference herein in its entirety fully as disclosed in the foregoing publication. Acid and base titrations over the pH ranges of 4.5 to 5.0 and 5.0 to 5.5, and the calculation of buffer capacity in this range of an IL-4 receptor specific antibody ("Ab-hIL4R") are described in the Examples below.

Interleukin 1 - receptor 1 ("IL1-R1") specific antibodies, peptibodies and related proteins and the like, including but not limited to those described in U.S. Application Publication Number US2004/097712A1 which is incorporated herein by reference in its entirety in parts pertinent to IL1-R1 specific binding proteins, monoclonal antibodies in particular, especially, without limitation, those designated therein: 15CA, 26F5, 27F2, 24E12, and 10H7, each of which is individually and specifically incorporated by reference herein in its entirety fully as disclosed in the aforementioned U.S. application publication.

Ang2 specific antibodies and peptibodies and related proteins and the like, including but not limited to those described in International Publication Number WO 03/057134 and U.S. Application Publication Number US2003/0229023, each of which is incorporated herein by reference in its entirety particularly in parts pertinent to Ang2 specific antibodies and peptibodies and the like, especially those of sequences described therein and including but not limited to: L1(N); L1(N) WT; L1(N) 1K WT; 2xL1(N); 2xL1(N) WT; Con4 (N), Con4 (N) 1K WT, 2xCon4 (N) 1K; L1(C); L1(C) 1K; 2xL1 (C); Con4 (C); Con4 (C) 1K; 2xCon4 (C) 1K; Con4-L1 (N); Con4-L1 (C); TN-12-9 (N); C17 (N); TN8-8(N); TN8-14 (N); Con 1 (N), also including anti-Ang 2 antibodies and formulations such as those described in International Publication Number WO 2003/030833 which is incorporated herein by reference in its entirety as to the same, particularly Ab526; Ab528; Ab531; Ab533; Ab535; Ab536; Ab537; Ab540; Ab543; Ab544; Ab545; Ab546; A551; Ab553; Ab555; Ab558; Ab559; Ab565; AbF1AbFD; AbFE; AbFJ; AbFK; AbG1D4; AbGC1E8; AbH1C12; AblA1; AblF; AblKAblP; and AblP, in their various permutations as described therein, each of which is individually and specifically incorporated by reference herein in its entirety fully as disclosed in the foregoing publication.

NGF specific antibodies, including, in particular, but not limited to those described in US Application Publication Number US2005/0074821, which is incorporated herein by reference in its entirety particularly as to NGF-specific

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antibodies and related proteins in this regard, including in particular, but not limited to, the NGF-specific antibodies therein designated 4D4, 4G6, 6H9, 7H2, 14D10 and 14D11, each of which is individually and specifically incorporated by reference herein in its entirety fully as disclosed in the foregoing publication.

CD22 specific antibodies and related proteins, such as those described in US 5,789,554 which is incorporated herein by reference in its entirety as to CD22 specific antibodies and related proteins, particularly human CD22 specific antibodies, such as but not limited to humanized and fully human antibodies, including but not limited to humanized and fully human monoclonal antibodies, particularly including but not limited to human CD22 specific IgG antibodies, such as, for instance, a dimer of a human-mouse monoclonal hLL2 gamma-chain disulfide linked to a human-mouse monoclonal hLL2 kappa-chain, including, but limited to, for example, the human CD22 specific fully humanized antibody in Epratuzumab, CAS registry number 501423-23-0. Illustrative of the invention, acid and base titrations of a CD22-specific antibody ("Ab-hCD22") over the pH ranges of 4.5 to 5.0 and 5.0 to 5.5 are described in the Examples below. The calculation of buffer capacity of Ab-hCD22 in these pH ranges also is described in the Examples below.

IGF-1 receptor specific antibodies and related proteins such as those described in International Patent Application Number PCT/US2005/046493, which is incorporated herein by reference in its entirety as to IGF-1 receptor specific antibodies and related proteins, including but not limited to the IGF-1 specific antibodies therein designated L1H1, L2H2, L3H3, L4H4, L5H5, L6H6, L7H7, L8H8, L9H9, L10H10, L11H11, L12H12, L13H13, L14H14, L15H15, L16H16, L17H17, L18H18, L19H19, L20H20, L21H21, L22H22, L23H23, L24H24, L25H25, L26H26, L27H27, L28H28, L29H29, L30H30, L31H31, L32H32, L33H33, L34H34, L35H35, L36H36, L37H37, L38H38, L39H39, L40H40, L41H41, L42H42, L43H43, L44H44, L45H45, L46H46, L47H47, L48H48, L49H49, L50H50, L51H51, and L52H52, each of which is individually and specifically incorporated by reference herein in its entirety fully as disclosed in the foregoing International Application.

B-7 related protein 1("B7RP-1") specific antibodies, (B7RP-1 also is referred to in the literature as B7H2, ICOSL, B7h, and CD275) particularly B7RP-specific fully human monoclonal IgG2 antibodies, particularly fully human IgG2 monoclonal antibody that binds an epitope in the first immunoglobulin-like domain of B7RP-1, especially those that inhibit the interaction of B7RP-1 with its natural receptor, ICOS,

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on activated T cells in particular, especially, in all of the foregoing regards, those disclosed in U.S. Provisional Application Number 60/700,265, filed 18 July 2005, which is incorporated herein by reference in its entirety as to such antibodies and related proteins, including but not limited to antibodies designated therein as follow: 16H (having light chain variable and heavy chain variable sequences SEQ ID NO:1 and SEQ ID NO:7 respectively therein); 5D (having light chain variable and heavy chain variable sequences SEQ ID NO:2 and SEQ ID NO:9 respectively therein); 2H (having light chain variable and heavy chain variable sequences SEQ ID NO:3 and SEQ ID NO:10 respectively therein); 43H (having light chain variable and heavy chain variable sequences SEQ ID NO:6 and SEQ ID NO:14 respectively therein); 41H (having light chain variable and heavy chain variable sequences SEQ ID NO:5 and SEQ ID NO:13 respectively therein); and 15H (having light chain variable and heavy chain variable sequences SEQ ID NO:4 and SEQ ID NO:12 respectively therein), each of which is individually and specifically incorporated by reference herein in its entirety fully as disclosed in the foregoing U.S. Provisional Application. Acid and base titrations and determination of buffer capacity of a B7RP-1 specific antibody ("Ab-hB7RP1") are illustrated in the Examples below.

IL-15 specific antibodies, peptibodies and related proteins, such as, in particular, humanized monoclonal antibodies, particularly antibodies such as those disclosed in U.S. Application Publication Numbers: US2003/0138421; US2003/023586; US2004/0071702, each of which is incorporated herein by reference in its entirety as to IL-15 specific antibodies and related proteins, including peptibodies, including particularly, for instance, but not limited to, HuMax IL-15 antibodies and related proteins, such as, for instance, 146B7.

IFN gamma specific antibodies, especially human IFN gamma specific antibodies, particularly fully human anti-IFN gamma antibodies, such as, for instance, those described in US Application Publication Number US2005/0004353, which is incorporated herein by reference in its entirety as to IFN gamma specific antibodies, particularly, for example, the antibodies therein designated 1118; 1118*; 1119; 1121; and 1121* each of which is individually and specifically incorporated by reference herein in its entirety fully as disclosed in the foregoing US Application Publication.

TALL-1 specific antibodies and other TALL specific binding proteins such as those described in U.S. Application Publication Number 2003/0195156 which is incorporated herein by reference in its entirety as to TALL-1 binding proteins,

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particularly the molecules of Tables 4 and 5B, each of which is individually and specifically incorporated by reference herein in its entirety fully as disclosed in the foregoing US Application Publication.

Stem Cell Factor (s) ("SCF") and related proteins such as those described in U.S. Patent Numbers 6,204,363 and 6,207,802, each of which is incorporated herein by reference in its entirety as to stem cell factors and related proteins, particularly, for example, the stem cells factor "STEMGEN ™."

Flt3-Ligands, ("Flt3L") and related proteins such as those described in U.S. Patent Number 6,632,424 which is incorporated herein by reference as to Flt3-ligands and related proteins in this regard.

IL-17 receptors and related proteins ("IL-17R"), such as those described in U.S. Patent Number 6,072,033 which is incorporated herein by reference as to Flt3-ligands and related proteins in this regard.

Etanercept, also referred to as Embrel, and related proteins.

Actimmune (Interferon-gamma-1b), Activase (Alteplase), Aldurazme (Laronidase), Amevive (Alefacept), Avonex (Interferon beta-1a), BeneFIX (Nonacog alfa), Beromun (Tasonermin), Beatseron (Interferon-beta-1b), BEXXAR (Tositumomab), Tev-Tropin (Somatropin), Bioclate or RECOMBINATE (Recombinant), CEREZME (Imiglucerase), ENBREL (Etanercept), Eprex (epoetin alpha), EPOGEN/Procit (Epoetin alfa), FABRAZYME (Agalsidase beta), Fasturtec/Elitek ELITEK (Rasburicase), FORTEO (Teriparatide), GENOTROPIN (Somatropin), GlucaGen (Glucagon), Glucagon (Glucagon, rDNA origin), GONAL-F (follitropin alfa), KOGENATE FS (Octocog alfa), HERCEPTIN (Trastuzumab), HUMATROPE (SOMATROPIN), HUMIRA (Adalimumab), Insulin in Solution, INFERGEN® (Interferon alfacon-1), KINERET® (anakinra), Kogenate FS (Antihemophilic Factor), LEUKIN (SARGRAMOSTIM Recombinant human granulocyte-macrophage colony stimulating factor (rhuGM-CSF)), CAMPATH (Alemtuzumab), RITUXAN® (Rituximab), TNKase (Tenecteplase), MYLOTARG (gemtuzumab ozogamicin), NATRECOR (nesiritide), ARANESP (darbepoetin alfa), NEULASTA (pegfilgrastim), NEUMEGA (oprelvekin), NEUPOGEN (Filgrastim), NORDITROPIN CARTRIDGES (Somatropin), NOVOSEVEN (Eptacog alfa), NUTROPIN AQ (somatropin), Oncaspar (pegaspargase), ONTAK (denileukin diftitox), ORTHOCLONE OKT (muromonab-CD3), OVIDREL (choriogonadotropin alfa), PEGASYS (peginterferon alfa-2a), PROLEUKIN (Aldesleukin), PULMOZYME

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(dornase alfa), Retavase (Reteplase), REBETRON Combination Therapy containing REBETOL® (Ribavirin) and INTRON® A (Interferon alfa-2b), REBIF (interferon beta-1a), REFACTO (Antihemophilic Factor), REFLUDAN (lepirudin), REMICADE (infliximab), REOPRO (abciximab)ROFERON®-A (Interferon alfa-2a), SIMULECT (baasiliximab), SOMAVERT (Pegivisomant), SYNAGIS® (palivizumab), Stemben (Ancestim, Stem cell factor), THYROGEN, INTRON® A (Interferon alfa-2b), PEG-INTRON® (Peginterferon alfa-2b), XIGRIS® (Drotrecogin alfa activated), XOLAIR® (Omalizumab), ZENAPAX® (daclizumab), ZEVALIN® (Ibritumomab Tiuxetan).

d. Sequence Variation

Particularly preferred proteins in regard to all of the foregoing and the following, include those that comprise a region that is 70% or more, especially 80% or more, more especially 90% or more, yet more especially 95% or more, particularly 97% or more, more particularly 98% or more, yet more particularly 99% or more identical in amino acid sequence to a reference amino acid sequence of a binding protein, as illustrated above, particularly a pharmaceutical binding protein, such as a GenBank or other reference sequence of a reference protein.

Identity in this regard can be determined using a variety of well-known and readily available amino acid sequence analysis software. Preferred software includes those that implement the Smith-Waterman algorithms, considered a satisfactory solution to the problem of searching and aligning sequences. Other algorithms also may be employed, particularly where speed is an important consideration. Commonly employed programs for alignment and homology matching of DNAs, RNAs, and polypeptides that can be used in this regard include FASTA, TFASTA, BLASTN, BLASTP, BLASTX, TBLASTN, PROSRCH, BLAZE, and MPSRCH, the latter being an implementation of the Smith-Waterman algorithm for execution on massively parallel processors made by MasPar.

The BLASTN, BLASTX, and BLASTP programs are among preferred programs for such determinations, the former for polynucleotide sequence comparisons and the latter two for polypeptide sequence comparisons: BLASTX for comparison of the polypeptide sequences from all three reading frames of polynucleotide sequence and BLASTP for a single polypeptide sequence.

BLAST provides a variety of user definable parameters that are set before implementing a comparison. Some of them are more readily apparent than others on

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graphical user interfaces, such as those provided by NCBI BLAST and other sequence alignment programs that can be accessed on the internet. The settings and their values are set out and explained on the service web sites and are explained and set out in particular detail in a variety of readily available texts, including but not limited to BIOINFORMATICS: SEQUENCE AND GENOME ANALYSIS, 2nd Ed., David W. Mount, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (2004), especially Chapters 3, 4, 5, and 6 as to comparison of protein and nucleic acid sequences in general and as to BLAST comparisons and searches in particular; SEQUENCE ANALYSIS IN A NUTSHELL: A GUIDE TO COMMON TOOLS AND DATABASES, Scott Markel and Darryl León, O'Reilly & Associates, Sebastopol, California (2003), especially Chapter 7 as to BLAST in particular, each of which is herein incorporated by reference in its entirety particularly in parts pertinent to comparison of nucleotide and polypeptide sequences and to determining their degree of identity, similarity, homology and/or the like, especially as to comparison of a test sequence and a reference sequence to calculate a degree (percent) of identity between them.

In preferred embodiments of the invention in this regard, relatedness of sequences is defined as the identity score in percent returned by any one or another of the aforementioned BLAST comparison searches with e =10 and all other parameters set to their default values on the NCBI web server as set forth in SEQUENCE ANALYSIS IN A NUTSHELL: A GUIDE TO COMMON TOOLS AND DATABASES, Scott Markel and Darryl León, O'Reilly & Associates, Sebastopol, California (2003), pages 47-51 which are incorporated herein by reference in their entireties and in all particulars of the preferred settings for parameters of the present invention for comparing sequences using BLAST, such as those on NCBI BLAST.

The following references provide additional information on sequence comparisons in this regard, and in others. GUIDE TO HUMAN GENOME COMPUTING, Ed. Martin J. Bishop, Academic Press, Harcourt Brace & Company Publishers, New York (1994), which is incorporated herein by reference in its entirety with regard to the foregoing, particularly in parts pertinent to determining identity and or homology of amino acid or polynucleotide sequences, especially Chapter 7. The BLAST programs are described in Altschul et al., "Basic Local Alignment Research Tool," *J Mol Biol* 215: 403-410 (1990), which is incorporated by reference herein in its entirety. Additional information concerning sequence analysis and homology and

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identity determinations are provided in, among many other references well-known and readily available to those skilled in the art: NUCLEIC ACID AND PROTEIN SEQUENCE ANALYSIS: A PRACTICAL APPROACH, Eds. M. J. Bishop and C. J. Rawings, IRL Press, Oxford, UK (1987); PROTEIN STRUCTURE: A PRACTICAL APPROACH, Ed. T. E. Creighton, IRL Press, Oxford, UK (1989); Doolittle, R. F.: "Searching through sequence databases," *Met Enz.* 183: 99-110 (1990); Meyers and Miller: "Optimal alignments in linear space" *Comput. Applica. in Biosci* 4: 11-17 (1988); Needleman and Wunsch: "A general method applicable to the search for similarities in amino acid sequence of two proteins," *J Mol Biol* 48: 443-453 (1970) and Smith and Waterman "Identification of common molecular subsequences," *J Mol Biol* 147: 1950 et seq. (1981), each of which is incorporated herein by reference in its entirety with reference to the foregoing, particularly in parts pertinent to sequence comparison and identity and homology determinations.

Particularly preferred embodiments in this regard have 50% to 150% of the activity of the aforementioned reference protein, particularly highly preferred embodiments in this regard have 60% to 125% of the activity of the reference protein, yet more highly preferred embodiments have 75% to 110% of the activity of the reference protein, still more highly preferred embodiments have 85% to 125% the activity of the reference, still more highly preferred embodiments have 90% to 110% of the activity of the reference.

4. Formulations

Many reagents and methods conventionally employed for the formulation of protein pharmaceuticals can be used for the formulation of self-buffering protein compositions in accordance with various aspects and preferred embodiments of the invention. However, in self-buffering protein formulations in accordance with the invention, buffering is provided substantially entirely by the protein itself, not by a buffering agent, as is the case with conventional formulations. Moreover, self-buffering protein formulations in accordance with various aspects and preferred embodiments of the invention are substantially free of such buffering agents.

In many other respects, however, self-buffering protein compositions in accordance with various aspects and embodiments of the invention can be formulated using reagents and methods conventionally employed for the formulation of proteins, in particular, reagents and methods employed for the formulation of pharmaceuticals,

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including pharmaceuticals for veterinary and human use, especially those reagents and methods suitable for formulating protein pharmaceuticals for veterinary and especially for human use.

In accordance therewith, many methods and ingredients for formulating and using pharmaceuticals that are well-known and routine in the pertinent arts can be used in designing, making, and using self-buffering protein formulations in accordance with various aspects and preferred embodiments of the invention relating thereto. Such methods and ingredients are described in, to name just a few readily available references in this regard, REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY, 21st Ed.; Beringer *et al.* Editors, Lippincott, Williams & Wilkins, Philadelphia, PA (2005); ANSEL'S PHARMACEUTICAL DOSAGE FORMS AND DRUG DELIVERY SYSTEMS, 8th Ed., Allen *et al.*, Editors, Lippincott, Williams & Wilkins, Philadelphia, PA (2005); and PHARMACEUTICAL FORMULATION OF PEPTIDES AND PROTEINS, Sven Frokjaer and Lars Hovgaard, Editors, CRC Press, Boca Raton, Florida (2000), each of which is herein incorporated in its entirety particularly in parts pertinent to conventional ingredients and methods that may be used in self-buffering formulations of proteins in accordance with various aspects and preferred embodiments of the invention relating thereto.

Additional methods and ingredients that can be useful in this regard are disclosed in, among others, US 6,171,586; WO 2005/044854; US 6,288,030; US 6,267,958; WO 2004/055164; US 4,597,966; US 2003/0138417; US 6,252,055; US 5,608,038; US 6,875,432; US 2004/0197324; WO 02/096457; US 5,945,098; US 5,237,054; US 6,485,932; US 6,821,515; US 5,792,838; US 5,654,403; US 5,908,826; EP 0 804 163; and WO 2005/063291, each of which is incorporated herein by reference in its entirety particularly in parts pertinent to pharmaceutically acceptable self-buffering protein formulations in accordance with the invention.

Various specific aspects of the ingredients and specific types of formulations are further described below, by way of illustration. The description thus provided is not exhaustive of the methods and compositions possible for self-buffering protein formulations in accordance with the various aspects and embodiments of the invention, nor is it in any way exclusive.

In preferred embodiments of a variety of aspects of the invention, formulations of self-buffering proteins comprise a protein and a carrier, which also may be referred to herein variously, as the case may be, as one or more of: a vehicle, a primary vehicle,

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a diluent, a primary diluent, a primary carrier, a solvent and/or a primary solvent. In the broadest sense the carrier may be a gas, a liquid, or a solid, as suits the phase of the composition and/or its use(s). In some embodiments of the invention in this regard, the carrier is a solid, such as a powder in which a protein may be dispersed. In preferred embodiments in this regard, the carrier is a liquid, particularly a liquid in which the self-buffering protein is highly soluble, particularly at concentrations that provide the desired buffer capacity. Liquid carriers may be organic or non-organic. Preferably they are aqueous, most preferably they are largely or entirely comprised of pure water.

It will be appreciated that formulations for pharmaceutical use in accordance with various aspects and embodiments of the invention must be compatible with the processes and conditions to which they will be subjected, such as, for instance, sterilization procedures (generally applied before mixing with an active agent), and conditions during storage.

Almost invariably, formulations in accordance with numerous aspects and embodiments of the invention will contain additional ingredients including but not limited in any way to excipients and other pharmaceutical agents. Nevertheless, it is to be understood that formulations in accordance with the invention are self-buffering formulations in which the buffer capacity is provided substantially or entirely by the primary protein itself, as described elsewhere herein.

Formulations in accordance with various aspects and embodiments of the invention may contain, among others, excipients, as described below, including but not limited to ingredients for modifying, maintaining, or preserving, for example, osmolality, osmolarity, viscosity, clarity, color, tonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the formulations and/or primary polypeptide and/or protein.

Formulations will, of course, depend upon, for example, the particular protein being formulated, the other active agents, such as other pharmaceuticals, that will be comprised in the formulation, the intended route of administration, the method of administration to be employed, the dosage, the dosing frequency, and the delivery format, among others.

Formulations in accordance with certain of the preferred embodiments in various aspects of the invention provide compositions comprising a protein preferably a pharmaceutical protein and a solvent, the protein having a buffer capacity per unit volume of at least that of approximately: 2.0 or 3.0 or 4.0 or 5.0 or 6.50 or 8.00 or 10.0

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or 15.0 or 20.0 or 30.0 or 40.0 or 50.0 or 75.0 or 100 or 125 or 150 or 200 or 250 or 300 or 350 or 400 or 500 or 700 or 1,000 or 1,500 or 2,000 or 2,500 or 3,000 or 4,000 or 5,000 mM sodium acetate buffer as determined over the range of pH 5.0 to 4.0 pH or 5.0 to 5.5 as described in Example 1 or 2 and elsewhere herein.

Formulations in accordance with certain of the preferred embodiments in various aspects of the invention provide self-buffering protein compositions, particularly pharmaceutical protein compositions, wherein, exclusive of the buffer capacity of the protein, the buffer capacity per unit volume of the composition is equal to or less than that of 1.0 or 1.5 or 2.0 or 3.0 or 4.0 or 5.0 mM sodium acetate buffer as determined over the range of pH 5.0 to 4.0 or pH 5.0 to 5.5 as described in Example 1 or 2 and elsewhere herein.

Formulations in accordance with certain of the preferred embodiments in various aspects of the invention provide self-buffering protein compositions, particularly pharmaceutical protein compositions, comprising a protein and a solvent, wherein at the pH of the composition the buffer capacity of the protein is at least approximately: 1.00 or 1.50 or 1.63 or 2.00 or 3.00 or 4.00 or 5.00 or 6.50 or 8.00 or 10.0 or 15.0 or 20.0 or 30.0 or 40.0 or 50.0 or 75.0 or 100 or 125 or 150 or 200 or 250 or 300 or 350 or 400 or 500 or 700 or 1,000 or 1,500 or 2,000 or 2,500 or 3,000 or 4,000 or 5,000 mEq per liter and per change in pH of one pH unit.

Formulations in accordance with certain of the preferred embodiments in various aspects of the invention provide self-buffering protein compositions, particularly pharmaceutical protein compositions, comprising a protein and a solvent, wherein at the pH of the composition, exclusive of the protein, the buffer capacity per unit volume of the composition is equal to or less than that of a 0.50 or 1.00 or 1.50 or 2.00 or 3.00 or 4.00 or 5.00 or 6.50 or 8.00 or 10.0 or 20.0 or 25.0 mM acetate buffer as determined over the range of pH 5.0 to 4.0 or pH 5.0 to 5.5 as described in Example 1 or 2 and elsewhere herein.

Formulations in accordance with certain of the preferred embodiments in various aspects of the invention provide self-buffering protein compositions, particularly pharmaceutical protein compositions, comprising a protein and a solvent, wherein at a desired pH, the protein provides at least approximately 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 99.5% of the buffer capacity of the composition.

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Formulations in accordance with certain of the preferred embodiments in various aspects of the invention provide self-buffering protein compositions, particularly pharmaceutical protein compositions, comprising a protein and a solvent, A, wherein the concentration of the protein is between approximately: 20 and 400, or 20 and 300, or 20 and 250, or 20 and 200, or 20 and 150 mg/ml.

Formulations in accordance with certain of the preferred embodiments in various aspects of the invention provide self-buffering protein compositions, particularly pharmaceutical protein compositions, comprising a protein and a solvent, wherein the pH maintained by the buffering action of the protein is a pH between approximately: 3.5 and 8.0, or 4.0 and 6.0, or 4.0 and 5.5, or 4.5 and 5.5.

Formulations in accordance with certain of the preferred embodiments in various aspects of the invention provide self-buffering protein compositions, particularly pharmaceutical protein compositions, comprising a protein and a solvent, wherein the salt concentration is less than: 150 mM or 125 mM or 100 mM or 75 mM or 50 mM or 25 mM.

Formulations in accordance with certain of the preferred embodiments in various aspects of the invention provide self-buffering protein compositions, particularly pharmaceutical protein compositions, comprising a protein and a solvent, and further comprising one or more pharmaceutically acceptable salts; osmotic balancing agents (tonicity agents); anti-oxidants; antibiotics; antimycotics; bulking agents; lyoprotectants; anti-foaming agents; chelating agents; preservatives; colorants; analgesics; or additional pharmaceutical agents.

Formulations in accordance with certain of the preferred embodiments in various aspects of the invention provide self-buffering protein compositions, particularly pharmaceutical protein compositions, comprising a protein and a solvent, and further comprising one or more pharmaceutically acceptable polyols in an amount that is hypotonic, isotonic, or hypertonic, preferably approximately isotonic, particularly preferably isotonic, especially preferably any one or more of sorbitol, mannitol, sucrose, trehalose, or glycerol, particularly especially preferably approximately 5% sorbitol, 5% mannitol, 9% sucrose, 9% trehalose, or 2.5% glycerol, very especially in this regard 5% sorbitol, 5% mannitol, 9% sucrose, 9% trehalose, or 2.5% glycerol.

Formulations in accordance with certain of the preferred embodiments in various aspects of the invention provide self-buffering protein compositions,

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particularly pharmaceutical protein compositions, comprising a protein and a solvent, and further comprising one or more pharmaceutically acceptable surfactants, preferably one or more of polysorbate 20, polysorbate 80, other fatty acid esters of sorbitan, polyethoxylates, and poloxamer 188, particularly preferably polysorbate 20 or polysorbate 80, preferably approximately 0.001 to 0.1% polysorbate 20 or polysorbate 80, very preferably approximately 0.002 to 0.02% polysorbate 20 or polysorbate 80, especially 0.002 to 0.02% polysorbate 20 or polysorbate 80.

Formulations in accordance with certain of the preferred embodiments in various aspects of the invention provide self-buffering protein compositions, particularly pharmaceutical protein compositions, comprising a protein and a solvent, wherein the protein is a pharmaceutical agent and the composition is a sterile formulation thereof suitable for treatment of a veterinary or a human medical subject.

Also among formulations in accordance with various aspects and embodiments of the invention herein described are lyophilized compositions in accordance with the foregoing, particularly lyophilized compositions that when reconstituted provide a formulation as described above and elsewhere herein.

a. Excipients and Other Additional Ingredients

As discussed above, certain embodiments in accordance with aspects of the invention provide self-buffering protein compositions, particularly pharmaceutical protein compositions, that comprise, in addition to the protein, particularly a pharmaceutical protein, one or more excipients such as those illustratively described in this section and elsewhere herein. Excipients can be used in the invention in this regard for a wide variety of purposes, such as adjusting physical, chemical, or biological properties of formulations, such as adjustment of viscosity, and or processes of the invention to improve effectiveness and or to stabilize such formulations and processes against degradation and spoilage due to, for instance, stresses that occur during manufacturing, shipping, storage, pre-use preparation, administration, and thereafter.

A variety of expositions are available on protein stabilization and formulation materials and methods useful in this regard, such as Arakawa et al., "Solvent interactions in pharmaceutical formulations," *Pharm Res.* 8(3): 285-91 (1991); Kendrick *et al.*, "Physical stabilization of proteins in aqueous solution," in: RATIONAL DESIGN OF STABLE PROTEIN FORMULATIONS: THEORY AND PRACTICE, Carpenter and Manning, eds. *Pharmaceutical Biotechnology.* 13: 61-84

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(2002), and Randolph *et al.*, "Surfactant-protein interactions," *Pharm Biotechnol.* 13: 159-75 (2002), each of which is herein incorporated by reference in its entirety, particularly in parts pertinent to excipients and processes of the same for self-buffering protein formulations in accordance with the current invention, especially as to protein pharmaceutical products and processes for veterinary and/or human medical uses.

Various excipients useful in the invention are listed in Table 1 and further described below.

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Table 1: Types of Excipients and Their Functions

Type	Function	
	Liquids	Lyophilates
Tonicity Agents / Stabilizers	• Provides isotonicity to the formulation such that it is suitable for injection • Examples include polyols, salts, and amino acids • Help maintain the protein in a more compact state (polyols) • Minimize electrostatic, solution protein-protein interactions (salts)	• Stabilizers include cryo and lyoprotectants • Examples include polyols, sugars and polymers • Cryoprotectants protect proteins from freezing stresses • Lyoprotectants stabilize proteins in the freeze-dried state
Bulking Agents	• Not applicable	• Used to enhance product elegance and to prevent blowout • Provides structural strength to the lyo cake • Examples include mannitol and glycine
Surfactants	• Prevent/control aggregation, particle formation and surface adsorption of drug • Examples include polysorbate 20 and 80	• Employed if aggregation during the lyophilization process is an issue • May serve to reduce reconstitution times • Examples include polysorbate 20 and 80
Anti-oxidants	• Control protein oxidation	• Usually not employed, molecular reactions in the lyophilized cake are greatly retarded
Metal Ions / Chelating Agents	• A specific metal ion is included in a liquid formulation only as a co-factor • Divalent cations such as zinc and magnesium are utilized in suspension formulations • Chelating agents are used to inhibit heavy metal ion catalyzed reactions	• May be included if a specific metal ion is included only as a co-factor • Chelating agents are generally not needed in lyophilized formulations
Preservatives	• Important particularly for multi-dose formulations • Protects against microbial growth, • Example: benzyl alcohol	• For multi-dose formulations only • Provides protection against microbial growth in formulation • Is usually included in the reconstitution diluent (e.g. bWFI)

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i. Salts

Salts may be used in accordance with certain of the preferred embodiments of the invention to, for example, adjust the ionic strength and/or the isotonicity of a self-buffering formulation and/or to improve the solubility and/or physical stability of a self-buffering protein or other ingredient of a self-buffering protein composition in accordance with the invention.

As is well known, ions can stabilize the native state of proteins by binding to charged residues on the protein's surface and by shielding charged and polar groups in the protein and reducing the strength of their electrostatic interactions, attractive, and repulsive interactions. Ions also can stabilize the denatured state of a protein by binding to, in particular, the denatured peptide linkages (-CONH) of the protein. Furthermore, ionic interaction with charged and polar groups in a protein also can reduce intermolecular electrostatic interactions and, thereby, prevent or reduce protein aggregation and insolubility.

Ionic species differ significantly in their effects on proteins. A number of categorical rankings of ions and their effects on proteins have been developed that can be used in formulating self-buffering protein compositions in accordance with the invention. One example is the Hofmeister series, which ranks ionic and polar non-ionic solutes by their effect on the conformational stability of proteins in solution. Stabilizing solutes are referred to as "kosmotropic." Destabilizing solutes are referred to as chaotropic. Kosmotropes commonly are used at high concentrations (e.g., >1 molar ammonium sulfate) to precipitate proteins from solution ("salting-out"). Chaotropes commonly are used to denture and/or to solubilize proteins ("salting-in"). The relative effectiveness of ions to "salt-in" and "salt-out" defines their position in the Hofmeister series.

In addition to their utilities and their drawbacks (as discussed above) salts also are effective for reducing the viscosity of protein formulations and can be used in the invention for that purpose.

In order to maintain isotonicity in a parenteral formulation in accordance with preferred embodiments of the invention, improve protein solubility and/or stability, improve viscosity characteristics, avoid deleterious salt effects on protein stability and aggregation, and prevent salt-mediated protein degradation, the salt concentration in self-buffering formulations in accordance with various preferred embodiments of the invention are less than 150 mM (as to monovalent ions) and 150 mEq/liter for

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multivalent ions. In this regard, in certain particularly preferred embodiments of the invention, the total salt concentration is from about 75 mEq/L to about 140 mEq/L.

ii. Amino acids

Free amino acids can be used in protein formulations in accordance with various preferred embodiments of the invention as, to name a few, bulking agents, stabilizers and antioxidants. However, amino acids comprised in self-buffering protein formulations in accordance with the invention do not provide buffering action. For this reason, those with significant buffer capacity either are not employed, are not employed at any pH around which they have significant buffering activity, or are used at low concentration so that, as a result, their buffer capacity in the formulation is not significant. This is particularly the case for histidine and other amino acids that commonly are used as buffers in pharmaceutical formulations.

Subject to the foregoing consideration, lysine, proline, serine, and alanine can be used for stabilizing proteins in a formulation. Glycine is useful in lyophilization to ensure correct cake structure and properties. As a result it is a common ingredient in lyophilized formulations and reconstituted lyophilates, such as Neumega ®, Genotropin®, and Humatrope®. Arginine may be useful to inhibit protein aggregation, in both liquid and lyophilized formulations, such as Activase®, Avonex®, and Enbrel® liquid. Methionine is useful as an antioxidant.

iii. Polyols

Polyols include sugars, e.g., mannitol, sucrose, and sorbitol and polyhydric alcohols such as, for instance, glycerol and propylene glycol, and, for purposes of discussion herein, polyethylene glycol (PEG) and related substances. Polyols are kosmotropic. They are useful stabilizing agents in both liquid and lyophilized formulations to protect proteins from physical and chemical degradation processes. Polyols also are useful for adjusting the tonicity of formulations.

Among polyols useful in the invention in this regard, is mannitol, commonly used to ensure structural stability of the cake in lyophilized formulations, such as, for example Leukine®, Enbrel® – Lyo, and Betaseron®. It ensures structural stability to the cake. It is generally used with a lyoprotectant, e.g., sucrose. Sorbitol and sucrose are among preferred agents for adjusting tonicity and as stabilizers to protect against freeze-thaw stresses during transport or the preparation of bulks during the manufacturing process. Reducing sugars (which contain free aldehyde or ketone groups), such as glucose and lactose, can glycate surface lysine and arginine residues.

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Therefore, they generally are not among preferred polyols for use in accordance with the invention. In addition, sugars that form such reactive species, such as sucrose, which is hydrolyzed to fructose and glucose under acidic conditions, and consequently engenders glycation, also is not among preferred amino acids of the invention in this regard. PEG is useful to stabilize proteins and as a cryoprotectant and can be used in the invention in this regard, such as it is in Recombinate®.

iv. Surfactants

Protein molecules are susceptible to adsorption on surfaces and to denaturation and consequent aggregation at air-liquid, solid-liquid, and liquid-liquid interfaces. These effects generally scale inversely with protein concentration. These deleterious interactions generally scale inversely with protein concentration and typically are exacerbated by physical agitation, such as that generated during the shipping and handling of a product.

Surfactants routinely are used to prevent, minimize, or reduce surface adsorption. Useful surfactants in the invention in this regard include polysorbate 20, polysorbate 80, other fatty acid esters of sorbitan polyethoxylates, and poloxamer 188.

Surfactants also are commonly used to control protein conformational stability. The use of surfactants in this regard is protein-specific since, any given surfactant typically will stabilize some proteins and destabilize others.

Polysorbates are susceptible to oxidative degradation and often, as supplied, contain sufficient quantities of peroxides to cause oxidation of protein residue side-chains, especially methionine. Consequently, polysorbates should be used carefully, and when used, should be employed at their lowest effective concentration. In this regard, polysorbates exemplify the general rule that excipients should be used in their lowest effective concentrations.

v. Antioxidants

A variety of processes can result in harmful oxidation of proteins in pharmaceutical formulations. To some extent deleterious oxidation of proteins can be prevented in pharmaceutical formulations by maintaining proper levels of ambient oxygen and temperature and by avoiding exposure to light. Antioxidant excipients can be used as well to prevent oxidative degradation of proteins. Among useful antioxidants in this regard are reducing agents, oxygen/free-radical scavengers, and chelating agents. Antioxidants for use in therapeutic protein formulations in accordance with the invention preferably are water-soluble and maintain their activity

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throughout the shelf life of a product. EDTA is a preferred antioxidant in accordance with the invention in this regard and can be used in the invention in much the same way it has been used in formulations of acidic fibroblast growth factor and in products such as Kineret® and Ontak®.

Antioxidants can damage proteins. For instance, reducing agents, such as glutathione in particular, can disrupt intramolecular disulfide linkages. Thus, antioxidants for use in the invention are selected to, among other things, eliminate or sufficiently reduce the possibility of themselves damaging proteins in the formulation.

vi. Metal Ions

Formulations in accordance with the invention may include metal ions that are protein co-factors and that are necessary to form protein coordination complexes, such as zinc necessary to form certain insulin suspensions. Metal ions also can inhibit some processes that degrade proteins. However, metal ions also catalyze physical and chemical processes that degrade proteins.

Magnesium ions (10 –120 mM) can be used to inhibit isomerization of aspartic acid to isoaspartic acid. Ca^{+2} ions (up to 100 mM) can increase the stability of human deoxyribonuclease (rhDNase, Pulmozyme®). Mg^{+2}, Mn^{+2}, and Zn^{+2}, however, can destabilize rhDNase. Similarly, Ca^{+2} and Sr^{+2} can stabilize Factor VIII, it can be destabilized by Mg^{+2}, Mn^{+2} and Zn^{+2}, Cu^{+2} and $Fe^{+2,}$ and its aggregation can be increased by Al^{+3} ions.

vii. Preservatives

Preservatives are necessary when developing multi-dose parenteral formulations that involve more than one extraction from the same container. Their primary function is to inhibit microbial growth and ensure product sterility throughout the shelf-life or term of use of the drug product. Commonly used preservatives include benzyl alcohol, phenol and m-cresol. Although preservatives have a long history of use with small-molecule parenterals, the development of protein formulations that includes preservatives can be challenging. Preservatives almost always have a destabilizing effect (aggregation) on proteins, and this has become a major factor in limiting their use in multi-dose protein formulations. To date, most protein drugs have been formulated for single-use only. However, when multi-dose formulations are possible, they have the added advantage of enabling patient convenience, and increased marketability. A good example is that of human growth hormone (hGH) where the development of preserved formulations has led to commercialization of more convenient, multi-use

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injection pen presentations. At least four such pen devices containing preserved formulations of hGH are currently available on the market. Norditropin® (liquid, Novo Nordisk), Nutropin AQ® (liquid, Genentech) & Genotropin (lyophilized – dual chamber cartridge, Pharmacia & Upjohn) contain phenol while Somatrope® (Eli Lilly) is formulated with m-cresol.

Several aspects need to be considered during the formulation and development of preserved dosage forms. The effective preservative concentration in the drug product must be optimized. This requires testing a given preservative in the dosage form with concentration ranges that confer anti-microbial effectiveness without compromising protein stability. For example, three preservatives were successfully screened in the development of a liquid formulation for interleukin-1 receptor (Type I) using differential scanning calorimetry (DSC). The preservatives were rank ordered based on their impact on stability at concentrations commonly used in marketed products.

As might be expected, development of liquid formulations containing preservatives are more challenging than lyophilized formulations. Freeze-dried products can be lyophilized without the preservative and reconstituted with a preservative containing diluent at the time of use. This shortens the time for which a preservative is in contact with the protein, significantly minimizing the associated stability risks. With liquid formulations, preservative effectiveness and stability have to be maintained over the entire product shelf-life (~ 18 to 24 months). An important point to note is that preservative effectiveness has to be demonstrated in the final formulation containing the active drug and all excipient components.

Self-buffering protein formulations in accordance with the invention, particularly self-buffering biopharmaceutical protein formulations, generally will be designed for specific routes and methods of administration, for specific administration dosages and frequencies of administration, for specific treatments of specific diseases, with ranges of bio-availability and persistence, among other things,

Formulations thus may be designed in accordance with the invention for delivery by any suitable route, including but not limited to orally, aurally, opthalmically, rectally, and vaginally, and by parenteral routes, including intravenous and intraarterial injection, intramuscular injection, and subcutaneous injection.

b. Formulations for Parenteral Administration

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Formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions and suspensions may be prepared from sterile powders or granules using one or more of the carriers or diluents mentioned for use in the formulations for oral administration or by using other suitable dispersing or wetting agents and suspending agents.

When parenteral administration is contemplated, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising the desired protein in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile pure water in which the protein is formulated as a sterile, isotonic self-buffering solution.

Such preparations may also involve the formulation of the desired protein in the form of, among other things, injectable microspheres, bio-erodible particles, polymeric compounds (polylactic acid, polyglycolic acid), beads, or liposomes, including those that provide for controlled or sustained release. Such formulations may be introduced by implantable drug delivery devices, among others.

Formulations for parenteral administration also may contain substances that adjust the viscosity. such as carboxymethyl cellulose, sorbitol, and dextran. Formulations may also contain ingredients that increase solubility of the desired protein or other ingredients and those that stabilize one or more such ingredients, including in some cases, the self-buffering protein.

c. Formulations for Pulmonary Administration

A pharmaceutical composition in accordance with certain embodiments of the invention may be suitable for inhalation. For pulmonary administration, the pharmaceutical composition may be administered in the form of an aerosol or with an inhaler including dry powder aerosol. For example, a binding agent may be formulated as a dry powder for inhalation. Inhalation solutions may also be formulated with a propellant for aerosol delivery. In yet another embodiment, solutions may be nebulized. Pulmonary administration is further described in PCT Application No. PCT/US94/001875, which describes pulmonary delivery of chemically modified proteins.

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d. Formulations for Oral Administration

For oral administration, the pharmaceutical composition may be in the form of, for example, a tablet, capsule, suspension, or liquid. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of the active ingredient. Examples of such dosage units are tablets or capsules. Formulations for oral administration in accordance with the invention in this regard can be made conventionally wherein buffering in the formulation is provided by the self-buffering protein as described elsewhere herein.

e. Controlled Release Formulations

Among additional formulations that can be useful in the invention as herein described are sustained- and controlled-delivery formulations. Techniques for making such sustained- and controlled-delivery formulations that may be used in accordance with various aspects and preferred embodiments of the invention are well-known to those skilled in the art. Among these are delivery methods that use liposome carriers, bio-erodible microparticles, porous beads, and semi-permeable polymer matrices, such as those described in PCT/US93/00829; U.S. 3,773,919; EP 58,481; Sidman et al., *Biopolymers*, 22:547-556 (1983); Langer et al., *J. Biomed. Mater. Res.*, 15:167-277, (1981); Langer et al., *Chem. Tech.*, 12:98-105(1982); EP 133,988; Eppstein et al., *Proc. Natl. Acad. Sci.* (USA), 82:3688-3692 (1985); EP 36,676; EP 88,046; and EP 143,949, each of which is hereby incorporated by reference in its entirety, particularly in parts pertinent to self-buffering sustained- and controlled-delivery pharmaceutical protein formulations in accordance with the invention herein described.

f. Sterilization

The pharmaceutical composition to be used for in vivo administration typically must be sterile. This may be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using this method may be conducted either prior to or following lyophilization and reconstitution. The composition for parenteral administration may be stored in lyophilized form or in solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

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g. Storage

Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (e.g., lyophilized) requiring reconstitution prior to administration.

h. Additional Pharmaceutical Agents

Self-buffering protein compositions in accordance with the invention, particularly self-buffering pharmaceutical protein compositions, can comprise in addition to the self-buffering protein of the composition, one or more additional pharmaceutical agents. Such agents may be proteins as well, or they may be other types of agents. Included among such agents are those for prevention or treatment of any disorder or disease. Such agents include, for instance, antibiotics and antimycotics. They also include agents for treating human disorders, including but not limited to, agents for treating inflammatory diseases, cancers, metabolic disorders, neurological and renal disorders, to name just a few. Agents that may be used in the invention in this regard also include agents useful to augment the action of a self-buffering composition and or prevent, ameliorate, or treat any undesirable side effects of the administration thereof.

i. Methods for Making Self-Buffering Protein Formulations

Compositions in accordance with the invention may be produced using well-known, routine methods for making, formulating, and using proteins, particularly pharmaceutical proteins. In certain of the preferred embodiments of a number of aspects of the invention in this regard, methods for preparing the compositions comprise the use of counter ions to remove residual buffering agents. In this regard the term counter ion is any polar or charged constituent that acts to displace buffer from the composition during its preparation. Counter ions useful in this regard include, for instance, glycine, chloride, sulfate, and phosphate. The term counter ion in this regard is used to mean much the same thing as displacement ion.

Residual buffering agents can be removed using the counter ions in this regard, using a variety of well-known methods, including but not limited to, standard methods of dialysis and high performance membrane diffusion-based methods such as tangential

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flow diafiltration. Methods for residual buffer removal employing a counter ion in this regard can also, in some cases, be carried out using size exclusion chromatography.

In certain related preferred embodiments in this regard, compositions in accordance with the invention are prepared by a process that involves dialysis against a bufferless solution at a pH below that of the preparation containing the self-buffering protein. In particularly preferred embodiments of the invention in this regard, the bufferless solution comprises counter ions, particularly those that facilitate removal of residual buffer and do not adversely affect the self-buffering protein or the formulation thereof. In further particularly preferred embodiments of the invention in this regard, following dialysis the pH of the preparation is adjusted to the desired pH using dilute acid or dilute base.

In certain related particularly preferred embodiments in this regard, compositions in accordance with the invention are prepared by a process that involves tangential flow diafiltration against a bufferless solution at a pH below that of the preparation containing the self-buffering protein. In particularly preferred embodiments of the invention in this regard, the bufferless solution comprises counter ions, particularly those that facilitate removal of residual buffer and do not adversely affect the self-buffering protein or the formulation thereof. In further particularly preferred embodiments of the invention in this regard, following diafiltration the pH of the preparation is adjusted to the desired pH using dilute acid or dilute base.

5. Routes of Administration

Formulations in accordance with the invention, in various embodiments, may be administered by a variety of suitable routes, well-known to those skilled in the art of administering therapeutics to a subject. In embodiments of the invention in this regard, one or more formulations, as described elsewhere herein, are administered via the alimentary canal. In other embodiments one or more formulations as described elsewhere herein are administered parenterally. In various embodiments one or more formulations may be administered via the alimentary canal in conjunction with one or more other formulations administered parenterally.

Such routes in a variety of embodiments include but are not limited to administration of the compositions orally, ocularly, mucosally, topically, rectally, pulmonarily, such as by inhalation spray, and epicutaneously. The following parenteral routes of administration also are useful in various embodiments of the invention:

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administration by intravenous, intraarterial, intracardiac, intraspinal, intrathecal, intraosseous, intraarticular, intrasynovial, intracutaneous, intradermal, subcutaneous, peritoneal, and/or intramuscular injection. In some embodiments intravenous, intraarterial, intracutaneous, intradermal, subcutaneous and/or intramuscular injection are used. In some embodiments intravenous, intraarterial, intracutaneous, subcutaneous, and/or intramuscular injection are used.

In certain embodiments of the invention the compositions are administered locally, for instance by intraocular injection to treat ocular neovascularization, retinopathy, or age-related macular degeneration.

6. Doses

The amount of a self-buffering protein formulation administered and the dosage regimen for treating a disease condition with the formulation depends on a variety of factors, including the age, weight, sex, and medical condition of the subject, the type of disease, the severity of the disease, the route and frequency of administration, and the particular formulation employed. In particular the amount will depend on the protein therapeutic being administered and any other therapeutic agents being administered in conjunction therewith. Dosages can be determined for formulations in accordance with the invention using well-established routine pharmaceutical procedures for this purpose.

7. Dosing Regimens

Formulations of the invention can be administered in dosages and by techniques well-known to those skilled in the medical and veterinary arts taking into consideration such factors as the age, sex, weight, and condition of the particular patient, and the formulation that will be administered (e.g., solid vs. liquid). Doses for humans or other mammals can be determined without undue experimentation by the skilled artisan, from this disclosure, the documents cited herein, and the knowledge in the art.

In accordance with various embodiments, proper dosages and dosing plans will depend on numerous factors, and may vary in different circumstances. The parameters that will determine the optimal dosage plans to be administered typically will include some or all of the following: the disease being treated and its stage; the species of the subject, their health, gender, age, weight, and metabolic rate; other therapies being

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administered; and expected potential complications from the subject's history or genotype.

The optimal dosing plan in a given situation also will take into consideration the nature of the formulation, the way it is administered, the distribution route following administration, and the rate at which it will be cleared both from sites of action and from the subject's body. Finally, the determination of optimal dosing preferably will provide an effective dose that is neither below the threshold of maximal beneficial effect nor above the threshold where the deleterious effects associated with the dose of the active agents outweighs the advantages of the increased dose.

It will be appreciated that a "dose" may be delivered all at once, fractionally, or continuously over a period of time. The entire dose also may be delivered to a single location or spread fractionally over several locations. Furthermore, doses may remain the same over a treatment, or they may vary.

In various embodiments, formulations in accordance with the invention are administered in an initial dose, and thereafter maintained by further administrations. A formulation of the invention in some embodiments is administered by one method initially, and thereafter administered by the same method or by one or more different methods. The dosages of on-going administrations may be adjusted to maintain at certain values the levels of the active agents in the subject. In some embodiments the compositions are administered initially, and/or to maintain their level in the subject, by intravenous injection. In a variety of embodiments, other forms of administration are used.

Formulations of the invention may be administered in many frequencies over a wide range of times, including any suitable frequency and range of times that delivers a treatment-effective dose. Doses may be continuously delivered, administered every few hours, one or more times a day, every day, every other day or several times a week, or less frequently. In some embodiments they are administered over periods of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more days. In some embodiments they are administered over periods of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or more months. In a variety of embodiments they are administered for a period of one, two, three, four, five, six, seven, eight, nine, ten, or more years. Suitable regimens for initial administration and further doses for sequential administrations may all be the same or may be variable. Appropriate regimens can be ascertained by the skilled artisan, from this disclosure, the

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documents cited herein, and the knowledge in the art. Generally lengths of treatment will be proportional to the length of the disease process, the effectiveness of the therapies being applied, and the condition and response of the subject being treated.

8. Diseases and Treatments

Self-buffering pharmaceutical protein compositions in accordance with the invention, in preferred embodiments, are useful to treat subjects suffering from a wide variety of disorders and diseases. As noted elsewhere herein, the invention provides, among others, self-buffering compositions of pharmaceutical antibodies, antibody-derived pharmaceutical proteins, and antibody-related pharmaceutical proteins, that can comprise Fc effector functions and binding domains specific for a wide variety of disease-related targets and that are useful for treating disease. These proteins and self-buffering compositions thereof are described at length herein above, as well as their use in treating various disorders and diseases associated with their targets. Methods for using the compositions, including formulation methods, administration methods, doses, and dosing methods are all described illustratively above. The formulation and administration of any particular composition of the invention can be tailored to the treatment of a particular disease, using well-known and routine techniques in the arts for doing so, taken in light of the guidance provided by the present description of the invention. Among diseases usefully treated using self-buffering pharmaceutical protein formulations in accordance with various aspects and preferred embodiments of the invention are inflammatory diseases, cancers, metabolic disorders, neurological and renal disorders, to name just a few.

9. Packaging and Kits

The invention also provides kits comprising self-buffering protein formulations, particularly kits comprising in one more containers, a self-buffering pharmaceutical protein formulation and instructions regarding the use thereof, particularly such kits wherein the formulation is a pharmaceutically acceptable formulation for human use. Among preferred kits are those comprising one or more containers of a self-buffering protein formulation of the invention and one or more separate documents, information pertaining to the contents of the kit, and/or the use of its contents, particularly those wherein the protein is a biopharmaceutical protein, especially those wherein the protein is a biopharmaceutical protein formulated for the treatment of a disease in humans.

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In certain aspects of the invention in this regard, preferred kits include kits as above further comprising one or more single or multi-chambered syringes (e.g., liquid syringes and lyosyringes) for administering one or more self-buffering protein formulations of the invention. In certain aspects of the invention in this regard, certain of the particularly preferred kits further comprise preloaded syringes. In further particularly preferred embodiments in this regard, the kits comprise a self-buffering pharmaceutical composition for parenteral administration, sealed in a vial under partial vacuum in a form ready for loading into a syringe and administration to a subject. In especially preferred embodiments in this regard, the composition is disposed therein under partial vacuum. In all of these regards and others, in certain further particularly preferred embodiments the kits contain one or more vials in accordance with any of the foregoing, wherein each vial contains a single unit dose for administration to a subject. In all these respects and others the invention further relates to kits comprising lyophilates, disposed as above, that upon reconstitution provide compositions in accordance therewith. In this regard, the invention further provides in certain of its preferred embodiments, kits that contain a lyophilate in accordance with the invention and a sterile diluent for reconstituting the lyophilate.

Examples

The present invention is additionally described by way of the following illustrative, non-limiting Examples.

EXAMPLE 1: Acid Titrations and Buffer Capacities of Sodium Acetate Buffers in the Range pH 5.0 to 4.0

A stock solution of known concentration of acetic acid was prepared by diluting ultrapure glacial acetic acid in HPLC grade water and then titrating the pH up to the desired value with NaOH. Stocks were equilibrated to the air and to 21° C. Volumetric standards were prepared at a concentration of 1 N and diluted as necessary with HPLC water.

One mM, 2.5 mM, 5 mM, 7.5 mM, 10 mM, and 15 mM sodium acetate buffers were prepared by diluting the stock in HPLC water. The solutions were titrated with HCl. 0.2 N HCl was used for the 1, 2.5, and 5 mM solutions, 0.4 N HCl was used for the 7.5 mM solution, and 0.8 N HCl was used for the 10 and 15 mM solutions. The titrations were performed using standard analytical laboratory techniques.

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Figure 1, Panel A shows the titration data and the least squares trend lines calculated from the data for each solution. The slope of the trend line calculated from each data set was taken as the buffer capacity of the corresponding acetate buffer. The linear dependence of buffer capacity on acetate buffer concentration is shown in Figure 1, Panel B.

EXAMPLE 2: Base Titrations and Buffer Capacities of Sodium Acetate Buffers in the Range pH 5.0 to 5.5

Acetate buffer stocks and solutions for titration were prepared as described in Example 1. The solutions were titrated as described in Example 1, except that the solutions were titrated from pH 5.0 to 5.5 and the titrations were done using NaOH instead of HCl. 0.2 N NaOH was used to titrate the 1, 2.5, and 5 mM solutions and 0.4 N NaOH was used for the 7.5, 10, and 15 mM solutions. The results of the titrations are shown in Figure 2A. The linear dependence of buffer capacity on concentration of acetate buffer is displayed in Figure 2B.

EXAMPLE 3: Determination of Acetate by HPLC

Acetate was determined in acetate buffer samples using analytical SE-HPLC. A standard curve for peak areas as a function of acetate concentration was established by analysis of acetate in buffers of known acetate concentration. The amount of acetate in test samples was interpolated from the standard curve. A standard curve is shown in Figure 3. Nominal and measured amount of acetate in test buffers are tabulated below the standard curve in the figure.

EXAMPLE 4: Acid Titrations of Ab-hOPGL Formulations Over the Range of pH 5.0 to pH 4.0

Bulk Ab-hOPGL in 10 mM acetate (nominal value), 5% sorbitol, pH 5.0 was diafiltered against 5.25% sorbitol, pH 3.2 (adjusted with HCl) in a LABSCALE TFF® system (Millipore) with a multi-manifold cassette, using 3 Millipore Pellicon XL 50 regenerated cellulose ultra-filtration membranes. The diafiltration solution was exchanged 8 to 10 times over the course of the diafiltration for each formulation. Following diafiltration, the pH of the resulting buffer-free solution was measured and adjusted to pH 5.0, using 0.05 N HCl or 0.05 N NaOH.

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One, 10, 30, 60, 90, and 110 mg/ml solutions were prepared for titration by dilution. The pH of each dilution was adjusted to pH 5.0 with NaOH or HCl as necessary. Titrations were carried out as described in the foregoing Examples. 0.2 N HCl was used to titrate the 1, 10, and 30 mg/ml solutions. 0.4 N HCl was used to titrate the 60 mg/ml solution. 0.8 N HCl was used to titrate the 90 and 110 solutions.

The results of the titrations are depicted in Figure 4. The least squares regression line is shown for the dataset for each concentration. The buffer capacity was taken as the slope of the regression line for each concentration.

EXAMPLE 5: Base Titrations of Ab-hOPGL Formulations Over the Range of pH 5.0 to 6.0

One, 10, 30, 60, 90, and 110 mg/ml solutions of Ab-hOPGL were prepared for titration as described in Example 4. Base titrations were carried out using NaOH as described in preceding Examples. 0.2 N NaOH was used for the 1, 10, 30, and 60 mg/ml solutions and 0.4 N NaOH was used for the 90 and 110 mg/ml solutions. Results of the titrations are depicted in the graph in Figure 5. Linear regression lines are shown for the data for each concentration. The buffer capacity was taken as the slope of the regression line for each concentration.

EXAMPLE 6: Residual Acetate Levels in Self-Buffering Ab-hOPGL Formulations

The amount of residual acetate was determined in Ab-hOPGL formulations using the methods described in Example 3. The results are depicted graphically in Figure 6, which shows a standard curve relating HPLC measurements to acetate concentrations and, below the graph, a tabulation of the results of determinations made on Ab-hOPGL formulations at different concentrations. Ab-hOPGL concentrations are indicated on the left ("Nominal") and the measured concentration of acetate in each of the Ab-hOPGL concentration is indicated on the right.

EXAMPLE 7: Buffer Capacity of Ab-hOPGL Formulations Plus or Minus Residual Acetate in the Range of pH 5.0 to 4.0

Self-buffered Ab-hOPGL formulations were prepared and titrated with HCl as described in foregoing Examples. In addition, data was adjusted by subtracting the contribution of residual acetate buffer based on the determination of acetate content by

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SE-HPLC as described in, for instance, Example 3. Buffer capacities were determined as described above. The same analysis was carried out on both sets of data. The results, depicted in Figure 7, show the effect of residual acetate on the buffer capacity of the Ab-hOPGL preparations. The results make it clear that the buffer capacity of residual acetate is a minor factor in the buffer capacity of the self-buffering Ab-hOPGL formulations that were analyzed.

EXAMPLE 8: Buffer Capacity of Ab-hOPGL Plus or Minus Residual Acetate in the Range of pH 5.0 to 6.0

Self-buffered Ab-hOPGL formulations were prepared and titrated with NaOH as described in foregoing Examples. In addition, data was adjusted by subtracting the contribution of residual acetate buffer based on the determination of acetate content by SE-HPLC as described in, for instance, Example 3. Buffer capacities were determined as described above. The same analysis was carried out on both sets of data. The results, depicted in Figure 8, show the effect of residual acetate on the buffer capacity of the Ab-hOPGL preparations. The results make it clear that the buffer capacity of residual acetate is a minor factor in the buffer capacity of the self-buffering Ab-hOPGL formulations that were analyzed.

EXAMPLE 9: pH and Ab-hOPGL Stability in Self-Buffered and Conventionally Buffered Formulations

Self-buffering formulations of Ab-hOPGL were prepared as described in the foregoing Examples. In addition, formulations were made containing a conventional buffering agent, either acetate or glutamate. All formulations contained 60 mg/ml Ab-hOPGL. The stability of pH and Ab-hOPGL in the formulations was monitored for six months of storage at 4° C. Stability was monitored by determining monomeric Ab-hOPGL in the formulations over the time course of storage. The determination was made using SE-HPLC as described above. The results for all three formulations are shown in Figure 9. Panel A shows the stability of Ab-hOPGL in the three formulations. Stability in the self-buffered formulation is as good as in the conventionally buffered formulations. Panel B shows the pH stability of the three formulations. Again, pH stability in the self-buffered formulation is as good as in the conventionally buffered formulations.

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EXAMPLE 10: Titration and Buffer Capacities of Ab-hB7RP1 – pH 5.0 to 4.0

Self-buffering formulations of Ab-hB7RP1 were prepared in concentrations of 1, 10, 30, and 60 mg/ml, as described for Ab-hOPGL in the foregoing Examples. Titrations were carried out using HCl as described above. In addition, data was adjusted by subtracting the contribution of residual acetate buffer based on the determination of acetate content by SE-HPLC as described in, for instance, Example 3. Figure 10, Panel A shows the titration results. Figure 10, Panel B shows the dependence of buffer capacity on the concentration of Ab-hB7RP1 formulations before and after subtracting the contribution of residual acetate buffer. The results clearly show the self-buffering capacity of Ab-hB7RP1 in this pH range. At 40 mg/ml it provides approximately as much buffer capacity in this pH range as 10 mM sodium acetate buffer. At 60 mg/ml it provides approximately as much buffer capacity as 15 mM sodium acetate buffer.

EXAMPLE 11: Titration and Buffer Capacities for Ab-hB7RP1 – pH 5.0 to 6.0

Self-buffering formulations of Ab-hB7RP1 were prepared in concentrations of 1, 10, 30, and 60 mg/ml, as described for Ab-hOPGL in the foregoing Examples. Titrations were carried out using NaOH as described above. In addition, data was adjusted by subtracting the contribution of residual acetate buffer based on the determination of acetate content by SE-HPLC as described in, for instance, Example 3. Figure 11, Panel A shows the titration results. Figure 11, Panel B shows the dependence of buffer capacity on the concentration of Ab-hB7RP1 formulations before and after subtracting the contribution of residual acetate buffer. The results clearly show the self-buffering capacity of Ab-hB7RP1 in this pH range. At 60 mg/ml it provides approximately as much buffer capacity in this pH range as 10 mM sodium acetate buffer.

EXAMPLE 12: Ab-hB7RP1 Stability in Self-Buffering and Conventionally Buffered Formulations at 4° C and 29° C

Ab-hB7RP1 was prepared as described in the foregoing Examples and formulated as described above, in self-buffering formulations and in formulations using a conventional buffering agent, either acetate or glutamate. All formulations contained 60 mg/ml Ab-hB7RP1. The stability of the solution's pH and of the Ab-hB7RP1 in the solution was monitored for twenty-six weeks of storage at 4° C or at 29° C. Stability

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was monitored by determining monomeric Ab-hB7RP1 in the formulations over the time course of storage. The determination was made using SE-HPLC as described above. The results are shown in Figure 12. Panel A shows the results for storage at 4° C. Panel B shows the results for storage at 29° C. Ab-hB7RP1 was at least as stable in the self-buffered formulation at 4° C as the conventionally buffered formulations. At 29° C the self-buffered formulation was at least as stable as the conventionally buffered formulations, and may have been slightly better from 10 weeks through the last time point.

EXAMPLE 13: pH Stability of Self-Buffered Ab-hB7RP1 at 4° C and 29° C

Self-buffered Ab-hB7RP1 at 60 mg/ml was prepared as described in the foregoing Example. pH was monitored over the time course and at the same temperatures as described therein. The results are shown in Figure 13.

EXAMPLE 14: Buffer Capacity of Ab-hCD22 Formulations – pH 4.0 to 6.0

Self-buffering formulations of Ab-hCD22 were prepared and titrated over the range of pH 5.0 to 4.0 and the range of 5.0 to 6.0, as described for Ab-hOPGL and Ab-hB7RP1 in the foregoing Examples. Buffer capacities were calculated from the titration data, also as described above. Buffer capacity as a function of concentration is shown in Figure 14 for both pH ranges. Panel A shows the buffer capacity of the Ab-hCD22 formulations over the range of pH 5.0 to 4.0. Buffer capacity is linearally dependent on concentration, and an approximately 21 mg/ml formulation of Ab-hCD22 has a buffer capacity equal to that of 10 mM sodium acetate buffer pH 5.0, measured in the same way. Panel B shows the buffer capacity as a function of concentration over the pH range 5.0 to 6.0. In this range of pH an approximately 30 mg/ml formulation of Ab-hCD22 has a buffer capacity equal to that of 10 mM sodium acetate buffer pH 5.0, measured in the same way.

EXAMPLE 15: Titrations and Buffer Capacities of Ab-hIL4R Formulations – pH 5.0 to 4.0

Self-buffering formulations of Ab-hIL4R were prepared in concentrations of 1, 10, 25, and 90 mg/ml, as described for Ab-hOPGL in the foregoing Examples. Titrations were carried out using HCl as described above. Figure 15, Panel A shows the titration results. Figure 15, Panel B shows the dependence of buffer capacity on the

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concentration of Ab-hIL4R. The results clearly show the self-buffering capacity of Ab-hIL4R in this pH range. At approximately 75 mg/ml it provides as much buffer capacity in this pH range as 10 mM sodium acetate pH 5.0, measured in the same way.

EXAMPLE 16: Titrations and Buffer Capacities of Ab-hIL4R Formulations – pH 5.0 to 6.0

Self-buffering formulations of Ab-hIL4R were prepared in concentrations of 1, 10, 25, and 90 mg/ml, as described for Ab-hOPGL in the foregoing Examples. Titrations were carried out using NaOH as described above. Figure 16, Panel A shows the titration results. Figure 16, Panel B shows the dependence of buffer capacity on the concentration of Ab-hIL4R in this pH range. The results clearly show the self-buffering capacity of Ab-hIL4R in this pH range. At approximately 90 mg/ml it provides as much buffer capacity in this pH range as 10 mM sodium acetate pH 5.0, measured in the same way.

EXAMPLE 17: Ab-hIL4R and pH Stability in Acetate and Self-Buffered Ab-hIL4R Formulations at 37° C

Self-buffered and acetate buffered formulations of Ab-hIL4R at pH 5.0 and 70 mg/ml were prepared as described above. pH and Ab-hIL4R stability were monitored in the formulations for 4 weeks at 37° C. Ab-hIL4R stability was monitored by SE-HPLC as described above. The results are shown in Figure 17. Panel A shows that Ab-hIL4R is at least as stable in the self-buffered formulation as in the sodium acetate buffer formulation. Panel B shows that pH in the self-buffered formulation is as stable as in the sodium acetate buffer formulation.

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What is claimed:

1. A composition comprising a pharmaceutical protein, wherein at the pH of the composition, 21° C, one atmosphere, and equilibrium with ambient atmosphere, the protein has a buffer capacity per unit volume of at least that of approximately 4.0 mM sodium acetate buffer in pure water in the range of pH 5.0 to 4.0 or pH 5.0 to 5.5 under the same conditions, wherein further, exclusive of the buffer capacity of said protein, the buffer capacity per unit volume of the composition under the same conditions is no more than that of 2.0 mM sodium acetate buffer in pure water in the range of pH 5.0 to 4.0 or pH 5.0 to 5.5 under the same conditions, wherein the composition has been approved for pharmaceutical use by an authority legally empowered to grant such approval.

2. A composition comprising a pharmaceutical protein, wherein at the pH of the composition, 21° C, one atmosphere, and equilibrium with ambient atmosphere, the protein has a buffer capacity per unit volume of at least 1.50 mEq/liter-pH unit, wherein further, exclusive thereof, the buffer capacity per unit volume of the composition is less than 0.5 mEq/liter-pH unit, wherein the composition has been approved for pharmaceutical use by an authority legally empowered to grant such approval.

3. A composition according to claim 1, wherein the protein provides at least 80% of the buffer capacity of the composition.

4. A composition according to claim 3, wherein the concentration of the protein is between approximately 20 and 400 mg/ml.

5. A composition according to claim 4, wherein the pH maintained by the buffering action of the protein is between approximately 3.5 and 8.0.

6. A composition according to claim 5, wherein the pH maintained by the buffering action of the protein is between approximately 4 and 6.

7. A composition according to claim 5, further comprising one or more pharmaceutically acceptable salts, wherein the total salt concentration is less than 150 mM.

8. A composition according to claim 7, further comprising one or more pharmaceutically acceptable salts, wherein the total salt concentration is less than 100 mM.

9. A composition according to claim 5, further comprising one or more pharmaceutically acceptable polyols.

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10. A composition according to claim 9, wherein the polyol is one or more of sorbitol, mannitol, sucrose, trehalose, or glycerol.

11. A composition according to claim 5, further comprising one or more pharmaceutically acceptable surfactants.

12. A composition according to claim 11, wherein the surfactant is one or more of polysorbate 20, polysorbate 80, other fatty acid esters of sorbitan, polyethoxylates, and poloxamer 188.

13. A composition according to claim 9, further comprising one or more pharmaceutically acceptable surfactants.

14. A composition according to claim 1, further comprising one or more pharmaceutically acceptable: osmotic balancing agents; anti-oxidants; antibiotics; antimycotics; bulking agents; lyoprotectants; anti-foaming agents; chelating agents; preservatives; colorants; analgesics; or additional pharmaceutical agents.

15. A composition according to claim 5, further comprising one or more pharmaceutically acceptable: osmotic balancing agents; anti-oxidants; antibiotics; antimycotics; bulking agents; lyoprotectants; anti-foaming agents; chelating agents; preservatives; colorants; analgesics; or additional pharmaceutical agents.

16. A composition according to claim 7, further comprising one or more pharmaceutically acceptable: osmotic balancing agents; anti-oxidants; antibiotics; antimycotics; bulking agents; lyoprotectants; anti-foaming agents; chelating agents; preservatives; colorants; analgesics; or additional pharmaceutical agents.

17. A composition according to any of claims 1, 5, 7, 9, 11, 13, or 14, wherein the protein is or comprises: an antibody, Fab fragment, Fab_2 fragment, Fab_3 fragment, Fc fragment, scFv fragment, bis-scFv(s) fragment, minibody, diabody, triabody tetrabody, VhH domain, V-NAR domain, V_H domain, V_L domain, camel Ig, Ig NAR, receptibody, peptibody, or a variant or a derivative thereof or a protein related thereto, or a modification thereof.

18. A composition according to claim 17, wherein the protein comprises an Fc fragment or a part thereof, or a variant or a derivative of an Fc fragment or a part thereof or a protein related to an Fc fragment or part thereof, or a modification of any thereof.

19. A composition according to claim 18, wherein the protein further comprises a first binding moiety of a pair of cognate binding moieties.

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20. A composition according to any of claims 1, 5, 7, 9, 11, 13, or 14, wherein the protein is selected from the group consisting of proteins that bind specifically to one or more CD proteins, HER receptor family proteins, cell adhesion molecules, growth factors, nerve growth factors, fibroblast growth factors, transforming growth factors (TGF), insulin-like growth factors, osteoinductive factors, insulins and insulin-related proteins, coagulation and coagulation-related proteins, colony stimulating factors (CSFs), other blood and serum proteins blood group antigens; receptors, receptor-associated proteins, growth hormone receptors, T-cell receptors; neurotrophic factors. neurotrophins, relaxins, interferons, interleukins, viral antigens, lipoproteins, integrins, rheumatoid factors, immunotoxins, surface membrane proteins, transport proteins, homing receptors, addressins, regulatory proteins, and immunoadhesins,

21. A composition according to any of claims 1, 5, 7, 9, 11, 13, or 14, wherein the protein is selected from the group consisting of: OPGL specific binding proteins, myostatin specific binding proteins, IL-4 receptor specific binding proteins, IL1-R1 specific binding proteins, Ang2 specific binding proteins, NGF-specific binding proteins, CD22 specific binding proteins, IGF-1 receptor specific binding proteins, B7RP-1 specific binding proteins, IFN gamma specific binding proteins, TALL-1 specific binding proteins, stem cell factors, Flt-3 ligands, and IL-17 receptors.

22. A composition according to any of claims 1, 5, 7, 9, 11, 13 or 14, wherein the protein is selected from the group consisting of proteins that bind specifically to one ormore of: CD3, CD4, CD8, CD19, CD20, CD34; HER2, HER3, HER4, the EGF receptor; LFA-1, Mol, p150,95, VLA-4, ICAM-1, VCAM, alpha v/beta 3 integrin; vascular endothelial growth factor ("VEGF"); growth hormone, thyroid stimulating hormone, follicle stimulating hormone, luteinizing hormone, growth hormone releasing factor, parathyroid hormone, mullerian-inhibiting substance, human macrophage inflammatory protein (MIP-1-alpha), erythropoietin (EPO), NGF-beta, platelet-derived growth factor (PDGF), aFGF, bFGF, epidermal growth factor (EGF), TGF-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta4, TGF-beta5, IGF-I, IGF-II, des(1-3)-IGF-I (brain IGF-I), insulin, insulin A-chain, insulin B-chain, proinsulin, insulin-like growth factor binding proteins;, such as, among others, factor VIII, tissue factor, von Willebrands factor, protein C, alpha-1–antitrypsin, plasminogen activators, such as urokinase and tissue plasminogen activator ("t-PA"), bombazine, thrombin, and thrombopoietin; M-CSF, GM-CSF, G-CSF, albumin, IgE, flk2/flt3

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receptor, obesity (OB) receptor, bone-derived neurotrophic factor (BDNF), NT-3, NT-4, NT-5, NT-6); relaxin A-chain, relaxin B-chain, prorelaxin; interferon-alpha, -beta, and -gamma;IL-1 to IL-10; AIDS envelope viral antigen; calcitonin, glucagon, atrial natriuretic factor, lung surfactant, tumor necrosis factor-alpha and -beta, enkephalinase, RANTES, mouse gonadotropin-associated peptide, Dnase, inhibin, and activin; protein A or D, bone morphogenetic protein (BMP), superoxide dismutase, decay accelerating factor (DAF).

23. A composition according to any of claims 1, 5, 7, 9, 11, 13 or 14, wherein the protein is selected from the group consisting of:Actimmune (Interferon-gamma-1b), Activase (Alteplase), Aldurazme (Laronidase), Amevive (Alefacept), Avonex (Interferon beta-1a), BeneFIX (Nonacog alfa), Beromun (Tasonermin), Beatseron (Interferon-beta-1b), BEXXAR (Tositumomab), Tev-Tropin (Somatropin), Bioclate or RECOMBINATE (Recombinant), CEREZME (Imiglucerase), ENBREL (Etanercept), Eprex (epoetin alpha), EPOGEN/Procit (Epoetin alfa), FABRAZYME (Agalsidase beta), Fasturtec/Elitek ELITEK (Rasburicase), FORTEO (Teriparatide), GENOTROPIN (Somatropin), GlucaGen (Glucagon), Glucagon (Glucagon, rDNA origin), GONAL-F (follitropin alfa), KOGENATE FS (Octocog alfa), HERCEPTIN (Trastuzumab), HUMATROPE (SOMATROPIN), HUMIRA (Adalimumab), Insulin in Solution, INFERGEN® (Interferon alfacon-1), KINERET® (anakinra), Kogenate FS (Antihemophilic Factor), LEUKIN (SARGRAMOSTIM Recombinant human granulocyte-macrophage colony stimulating factor (rhuGM-CSF)), CAMPATH (Alemtuzumab), RITUXAN® (Rituximab), TNKase (Tenecteplase), MYLOTARG (gemtuzumab ozogamicin), NATRECOR (nesiritide), ARANESP (darbepoetin alfa), NEULASTA (pegfilgrastim), NEUMEGA (oprelvekin), NEUPOGEN (Filgrastim), NORDITROPIN CARTRIDGES (Somatropin), NOVOSEVEN (Eptacog alfa), NUTROPIN AQ (somatropin), Oncaspar (pegaspargase), ONTAK (denileukin diftitox), ORTHOCLONE OKT (muromonab-CD3), OVIDREL (choriogonadotropin alfa), PEGASYS (peginterferon alfa-2a), PROLEUKIN (Aldesleukin), PULMOZYME (dornase alfa), Retavase (Reteplase), REBETRON Combination Therapy containing REBETOL® (Ribavirin) and INTRON® A (Interferon alfa-2b), REBIF (interferon beta-1a), REFACTO (Antihemophilic Factor), REFLUDAN (lepirudin), REMICADE (infliximab), REOPRO (abciximab)ROFERON®-A (Interferon alfa-2a), SIMULECT (baasiliximab), SOMAVERT (Pegivisomant), SYNAGIS® (palivizumab), Stemben (Ancestim, Stem cell factor), THYROGEN, INTRON® A (Interferon alfa-2b), PEG-

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INTRON® (Peginterferon alfa-2b), XIGRIS® (Drotrecogin alfa activated), XOLAIR® (Omalizumab), ZENAPAX® (daclizumab), and ZEVALIN® (Ibritumomab Tiuxetan).

24. A composition according to any of claims 1, 5, 7, 9, 11, 13, or 14, wherein the protein is Ab-hOPGL or a fragment thereof, or a variant or derivative of Ab-hOPGL or of a fragment thereof, or an Ab-hOPGL related protein or fragment thereof, or a modification of any thereof.

25. A composition according to any of claims 1, 5, 7, 9, 11, 13, or 14, wherein the protein is Ab-hOPGL.

26. A composition according to any of claims 1, 5, 7, 9, 11, 13, or 14, wherein the protein is Ab-hIL4R or a fragment thereof, or a variant or derivative of Ab-hIL4R or of a fragment thereof, or an Ab-hIL4R related protein or fragment thereof, or a modification of any thereof.

27. A composition according to any of claims 1, 5, 7, 9, 11, 13, or 14, wherein the protein is Ab-hIL4R.

28. A composition according to any of claims 1, 5, 7, 9, 11, 13, or 14, wherein the protein is Ab-hB7RP1 or a fragment thereof, or a variant or derivative of Ab-hB7RP1 or of a fragment thereof, or an Ab-hB7RP1 related protein or fragment thereof, or a modification of any thereof.

29. A composition according to any of claims 1, 5, 7, 9, 11, 13, or 14, wherein the protein is Ab-hB7RP1.

30. A lyophilate which upon reconstitution provides a composition according to any of claim 1, 5, 7, 9, 11, 13 or 14.

31. A kit comprising in one or more containers a composition according to any of claim 1, 5, 7, 9, 11, 13 or 14 and instructions regarding the use thereof.

32. A kit comprising in one or more containers a lyophilate according to claim 31, and instructions regarding the use thereof.

33. A method for treating a subject, comprising administering to a subject in an amount and by a route effective for treatment, a composition according to any of claim 1, 5, 7, 9, 11, 13 or 14.

34. A process for preparing a composition according to any of claim 1, 5, 7, 9, 11, 13 or 14, comprising removing residual buffer using a counter ion.

35. A process for preparing a composition according to claim 34, comprising removing residual buffer using any one or more of the following in the

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presence of a counter ion: size exclusion chromatography, dialysis, and/or tangential flow filtration.

36. A process for preparing a composition according to claim 35, comprising removing residual buffer using ion exchange chromatography.

37. A process for preparing a composition according to any of claim 1, 5, 7, 9, 11, 13 or 14, comprising removing residual buffer by diafiltration against a bufferless solution having a pH below the desired pH.

38. A process for preparing a composition according to claim 37, wherein following diafiltration the pH is adjusted to a desired pH by addition of dilute acid and/or dilute base.

39. A lyophilate which upon reconstitution provides a composition according to claim 20.

40. A kit comprising in one or more containers a composition according to claim 20 and instructions regarding the use thereof.

41. A kit comprising in one or more containers a lyophilate according to claim 40, and instructions regarding the use thereof.

42. A method for treating a subject, comprising administering to a subject in an amount and by a route effective for treatment, a composition according to claim 20.

43. A process for preparing a composition according to claim 20, comprising removing residual buffer using a counter ion.

44. A process for preparing a composition according to claim 43, comprising removing residual buffer using any one or more of the following in the presence of a counter ion: size exclusion chromatography, dialysis, and/or tangential flow filtration.

45. A process for preparing a composition according to claim 43, comprising removing residual buffer using ion exchange chromatography.

46. A process for preparing a composition according to claim 20, comprising removing residual buffer by diafiltration against a bufferless solution having a pH below the desired pH.

47. A process for preparing a composition according to claim 46, wherein following diafiltration the pH is adjusted to a desired pH by addition of dilute acid and/or dilute base.

48. A lyophilate which upon reconstitution provides a composition according to claim 21.

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49. A kit comprising in one or more containers a composition according claim 21 and instructions regarding the use thereof.

50. A kit comprising in one or more containers a lyophilate according to claim 49, and instructions regarding the use thereof.

51. A method for treating a subject, comprising administering to a subject in an amount and by a route effective for treatment, a composition according to claim 21.

52. A process for preparing a composition according to claim 21, comprising removing residual buffer using a counter ion.

53. A process for preparing a composition according to claim 52, comprising removing residual buffer using any one or more of the following in the presence of a counter ion: size exclusion chromatography, dialysis, and/or tangential flow filtration.

54. A process for preparing a composition according to claim 52, comprising removing residual buffer using ion exchange chromatography.

55. A process for preparing a composition according to claim 21, comprising removing residual buffer by diafiltration against a bufferless solution having a pH below the desired pH.

56. A process for preparing a composition according to claim 55, wherein following diafiltration the pH is adjusted to a desired pH by addition of dilute acid and/or dilute base.

57. A lyophilate which upon reconstitution provides a composition according to claim 23.

58. A kit comprising in one or more containers a composition according claim 23 and instructions regarding the use thereof.

59. A kit comprising in one or more containers a lyophilate according to claim 57, and instructions regarding the use thereof.

60. A method for treating a subject, comprising administering to a subject in an amount and by a route effective for treatment, a composition according to claim 23.

61. A process for preparing a composition according to claim 23, comprising removing residual buffer using a counter ion.

62. A process for preparing a composition according to claim 61, comprising removing residual buffer using any one or more of the following in the

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presence of a counter ion: size exclusion chromatography, dialysis, and/or tangential flow filtration.

63. A process for preparing a composition according to claim 61, comprising removing residual buffer using ion exchange chromatography.

64. A process for preparing a composition according to claim 21, comprising removing residual buffer by diafiltration against a bufferless solution having a pH below the desired pH.

65. A process for preparing a composition according to claim 62, wherein following diafiltration the pH is adjusted to a desired pH by addition of dilute acid and/or dilute base.

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Figure 1. Buffer Capacity of Acetate in the pH 4.0 - 5.0 Range

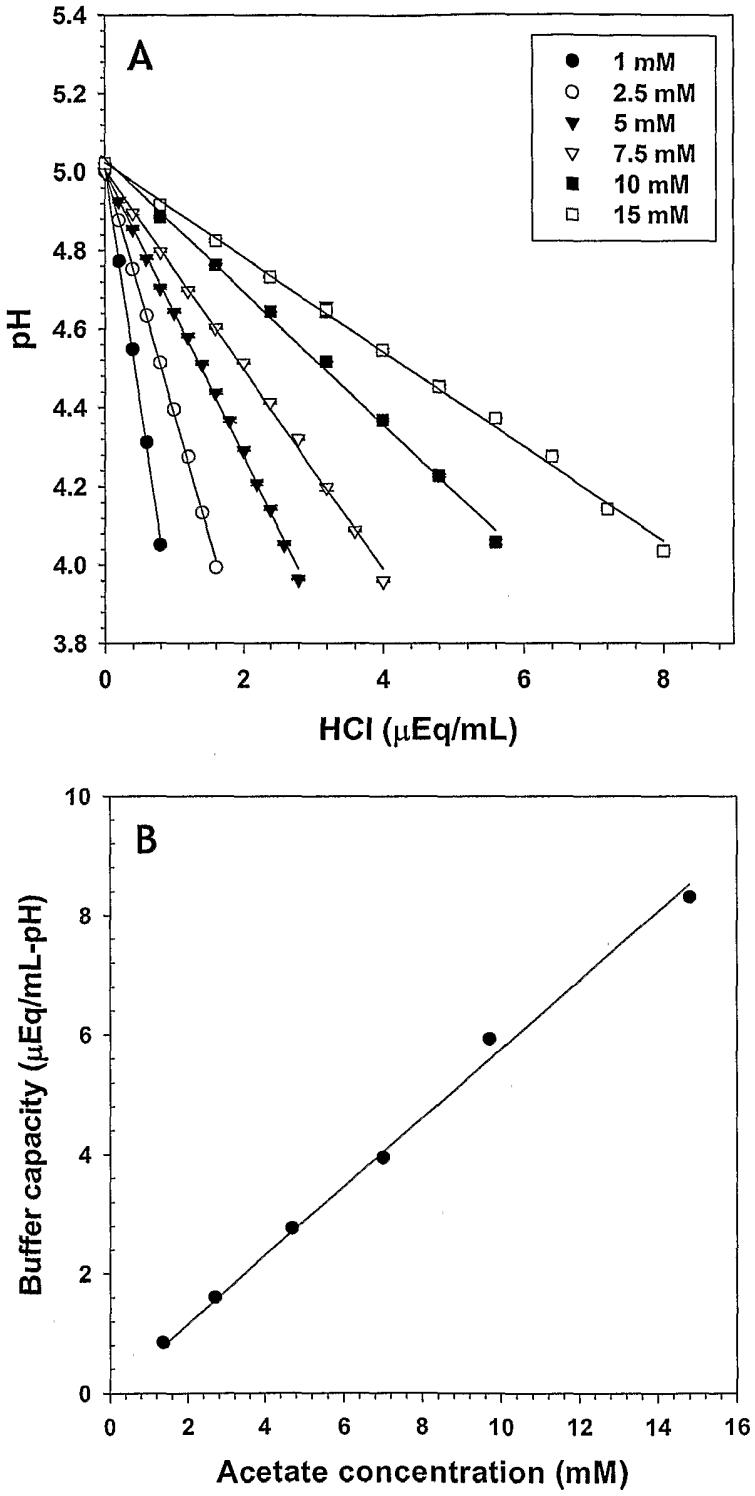


Figure 2. Buffer Capacity of Acetate in the pH 5.0 – 5.5 Range

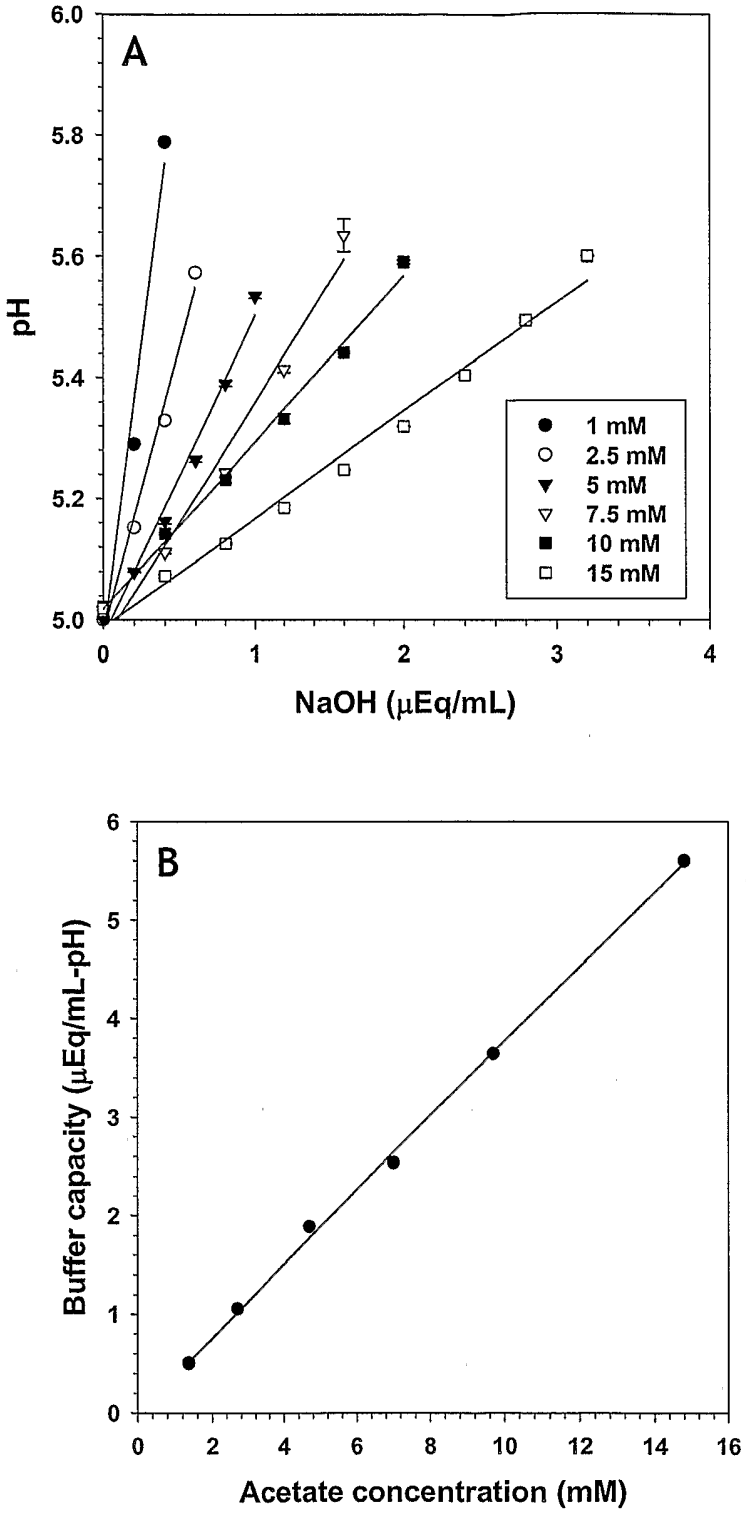
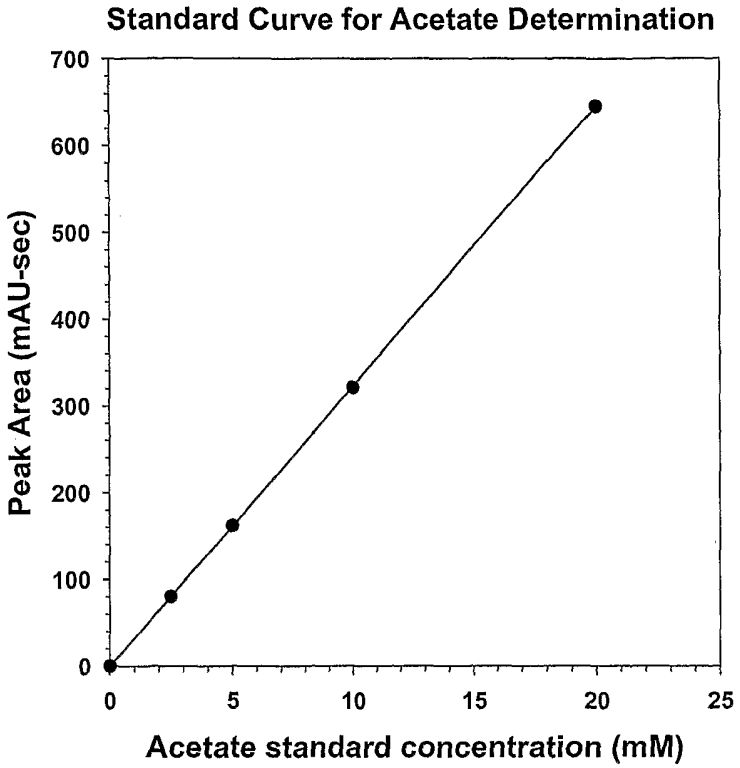


Figure 3. Determination of acetate concentration in acetate standards used for buffer capacity measurements



Experimentally determined acetate values for solutions used to determine acetate buffer capacity

Acetate concentration (mM)	
Nominal	Experimental
1	1.37
2.5	2.7
5	4.7
7.5	7.0
10	9.7
15	14.8

Figure 4. Acid titration of Ab-hOPGL Solutions

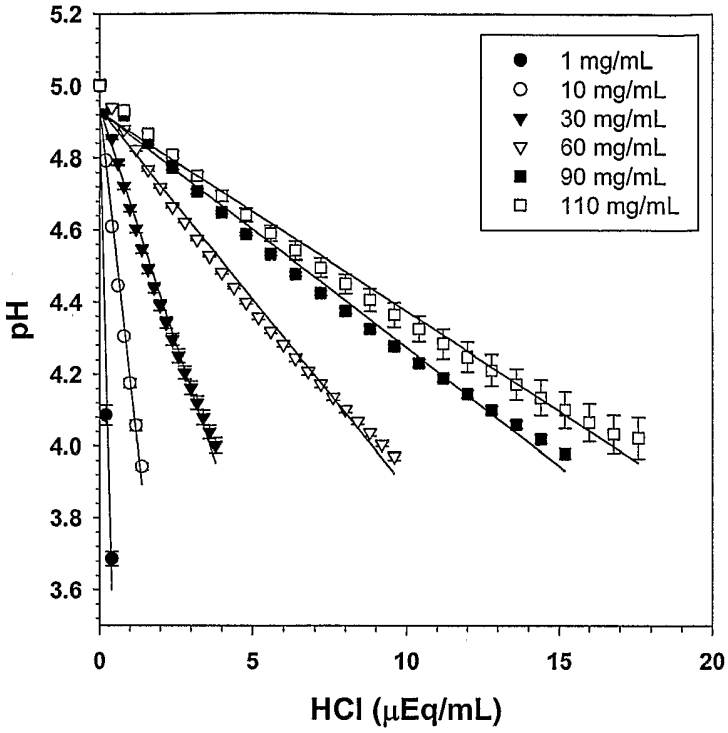


Figure 5. Base titration of Ab-hOPGL Solutions

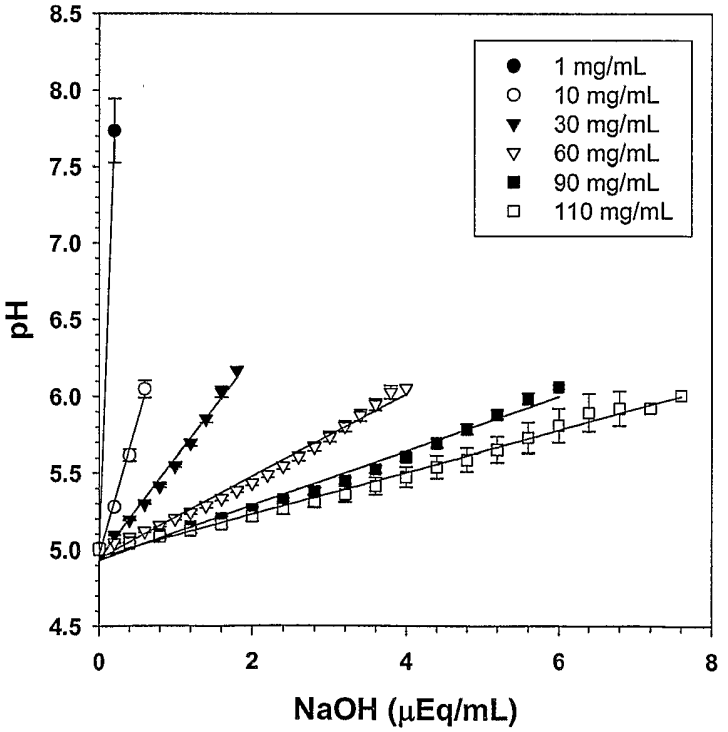
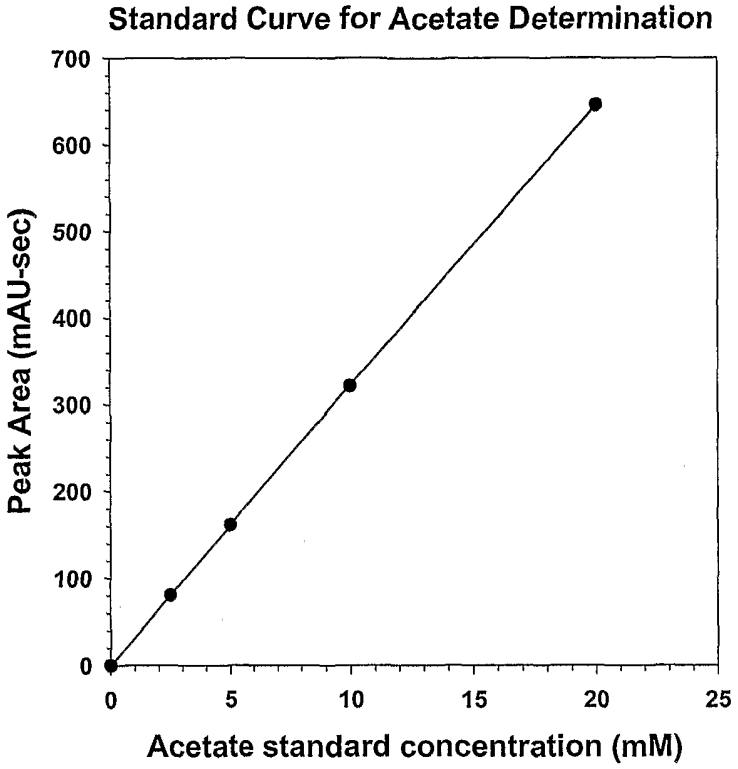


Figure 6. Determination of residual acetate levels in Ab-hOPGL Solutions for buffer capacity measurements



Residual acetate levels in Ab-hOPGL solutions used to determine buffer capacity

Acetate concentration (mM)	
Nominal	Experimental
1	0.05
10	0.20
30	0.51
60	1.16
90	1.71
110	1.82

Figure 7. Buffer capacity of Ab-hOPGL +/- residual acetate as a function of its concentration in the pH 4.0 – pH 5.0 range

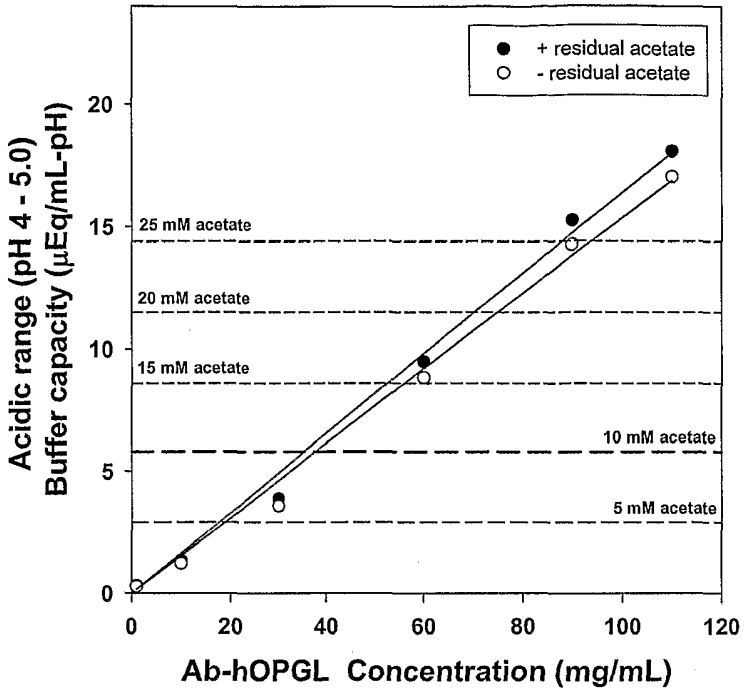


Figure 8. Buffer capacity of Ab-hOPGL +/- residual acetate as a function of its concentration in the pH 5.0 – pH 6.0 range

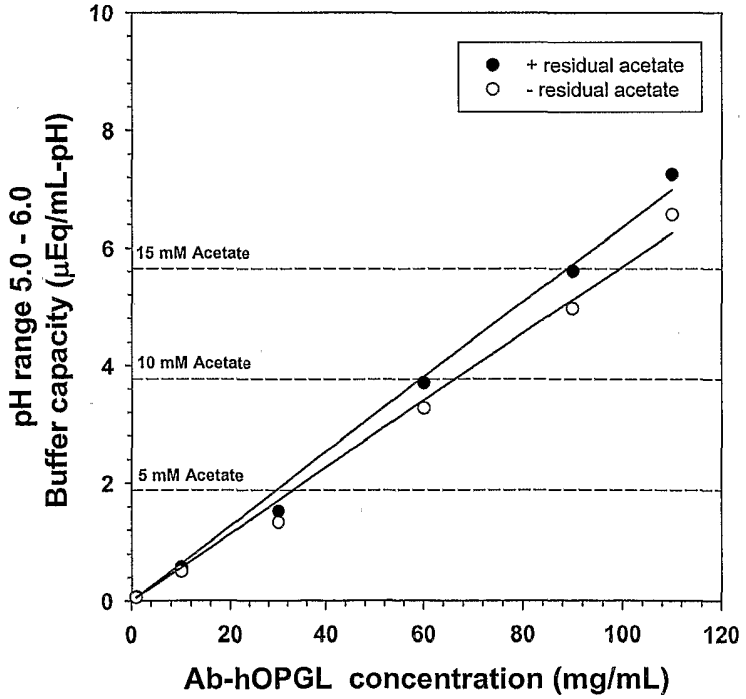


Figure 9. Stability of the Self-Buffered, 60 mg/mL, Ab-hOPGL Formulation Compared to Conventionally Buffered Formulations at 4 C

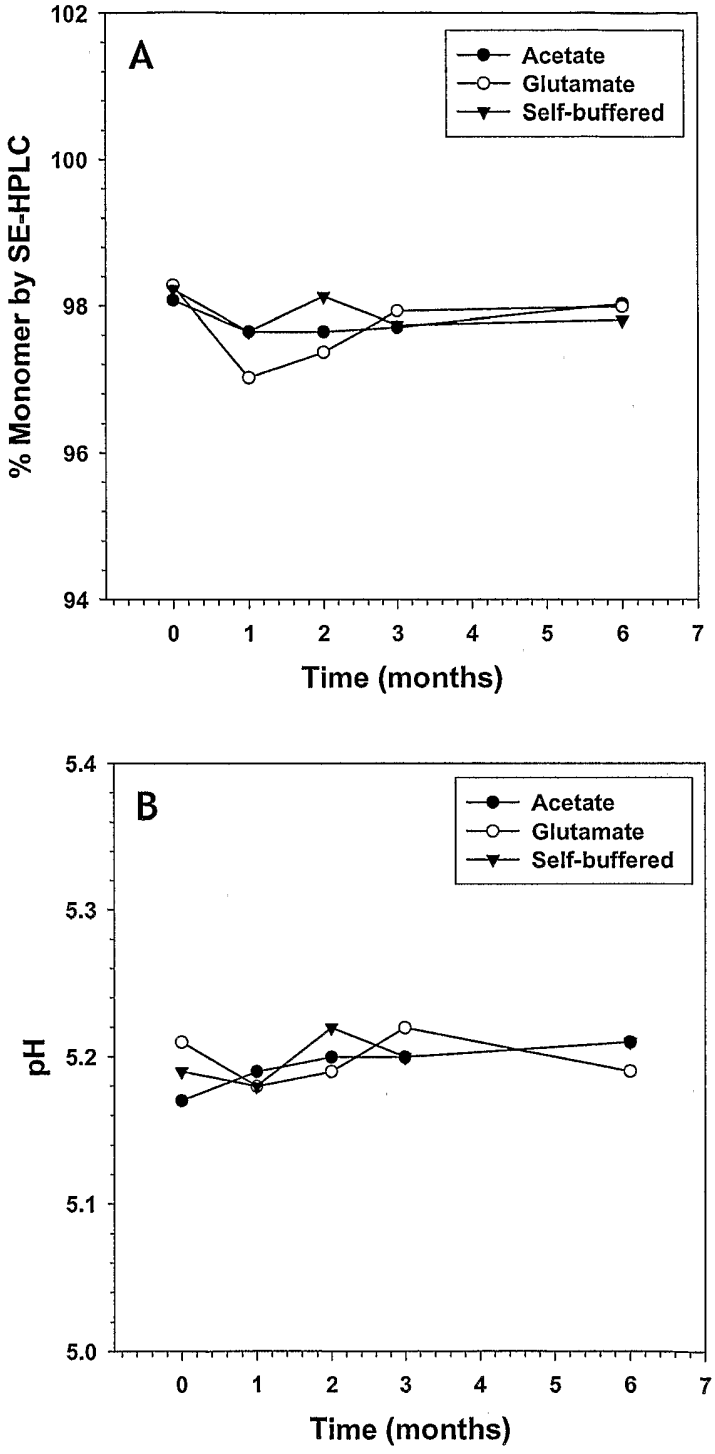
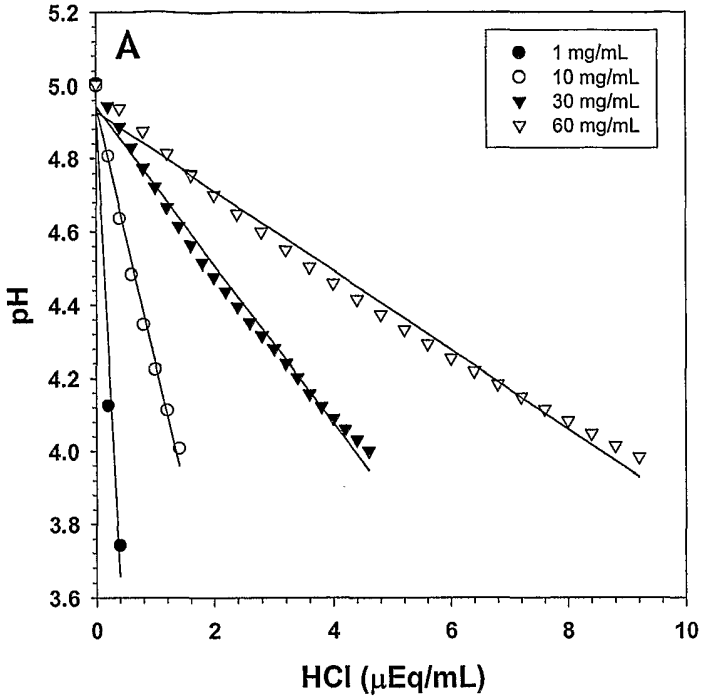


Figure 10. Buffer capacity of Ab-hB7RP1 Solutions in the pH 4.0 – 5.0 range



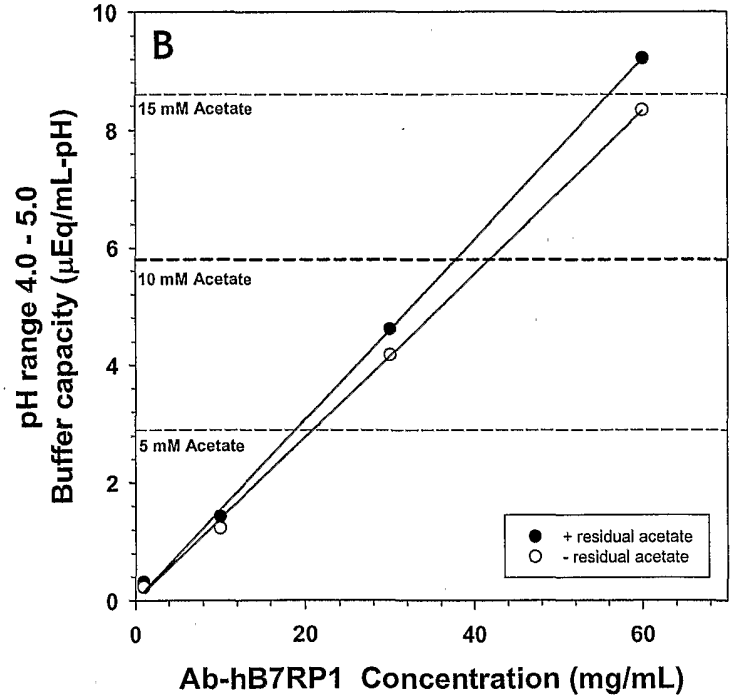


Figure 11. Buffer capacity of Ab-hB7RP1 Solutions in the pH 5.0-6.0 range

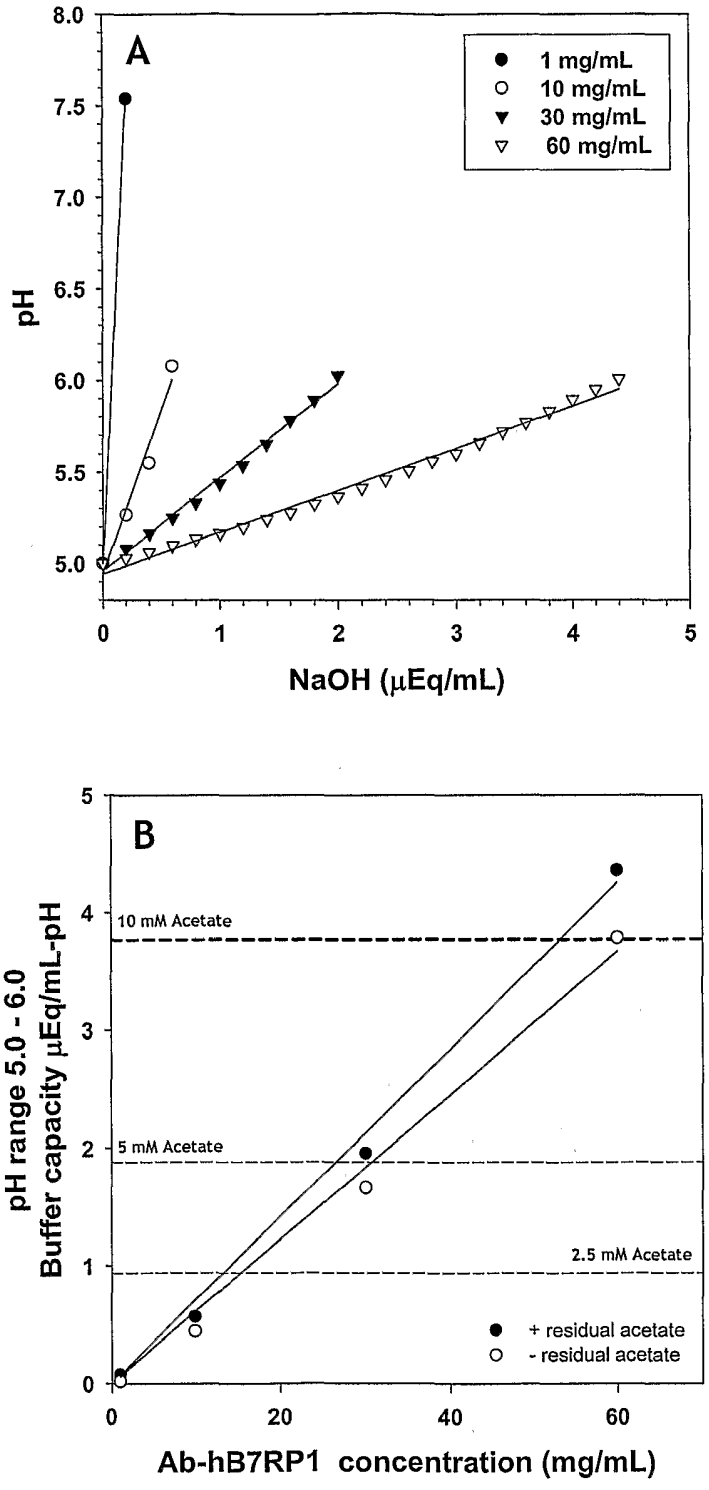


Figure 12. Stability of the Self-Buffered, 60 mg/mL, Ab-hB7RP1 Formulation Compared to Conventionally Buffered Formulations

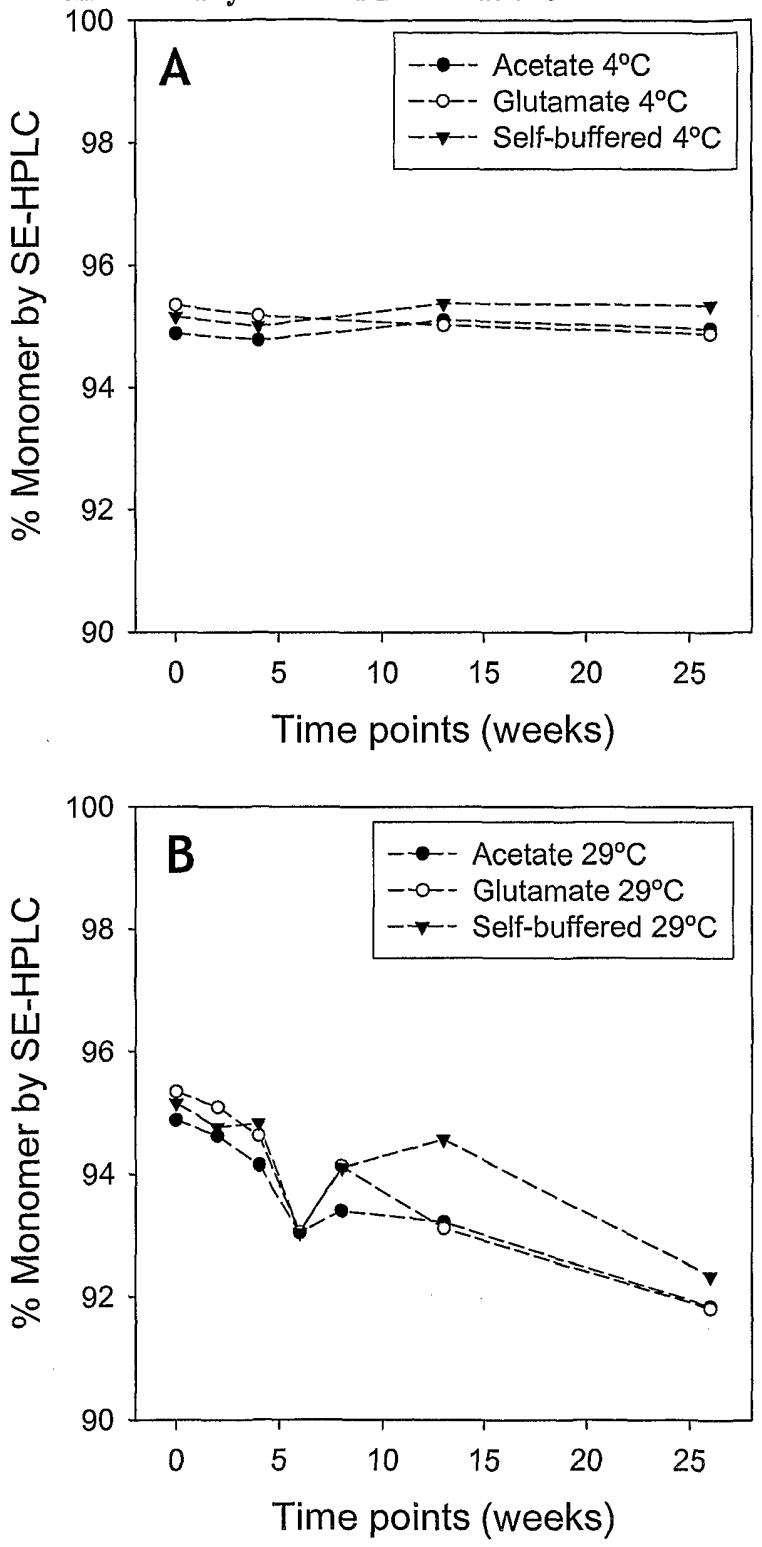


Figure 13. pH control of the Self-Buffered, 60 mg/mL, Ab-hB7RP1 Formulation

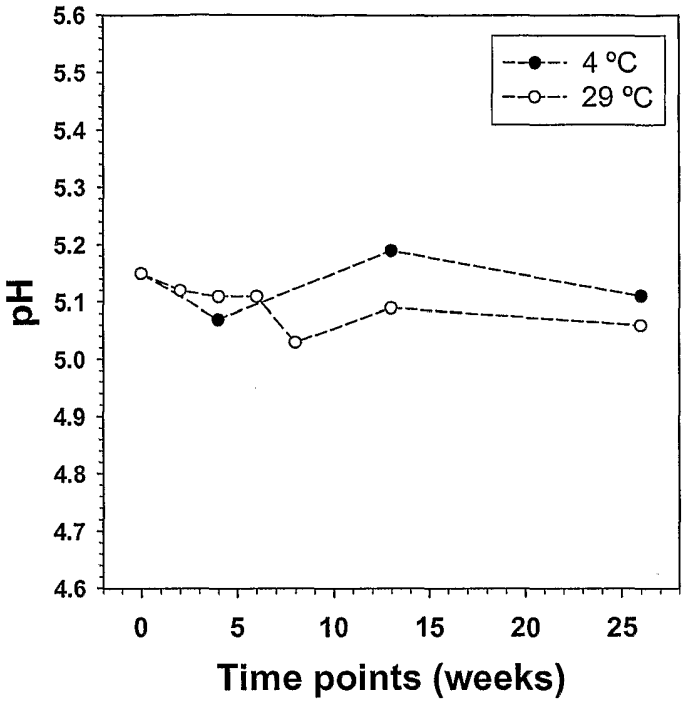
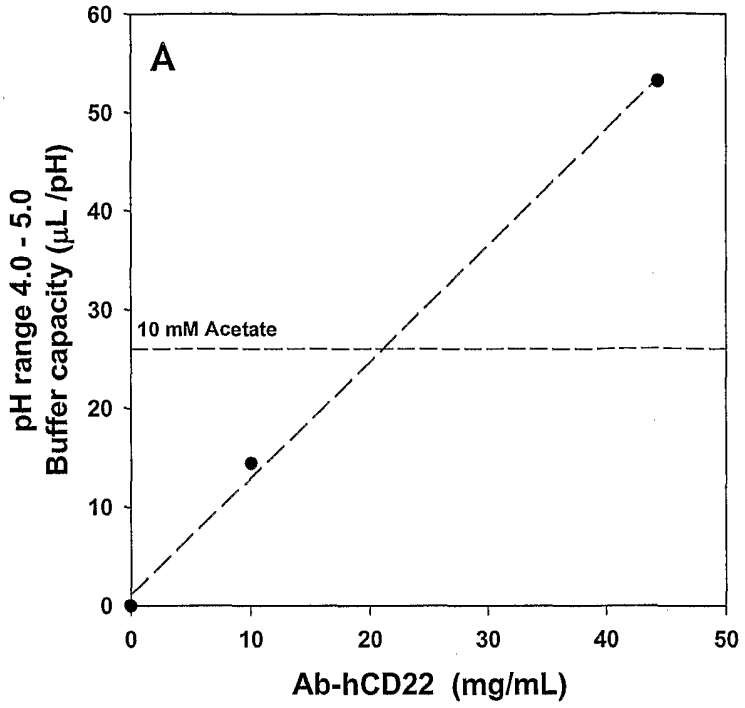


Figure 14. Buffer capacity of Ab-hCD22 solutions in the pH 4.0- 6.0 range



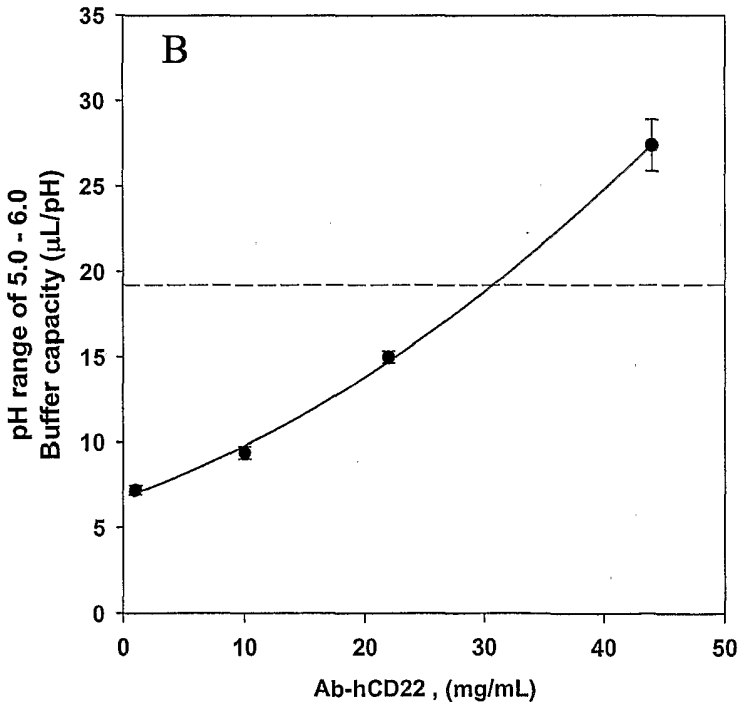


Figure 15. Buffer capacity of Ab-hIL4R Solutions in the pH 4.0 - 5.0 Range

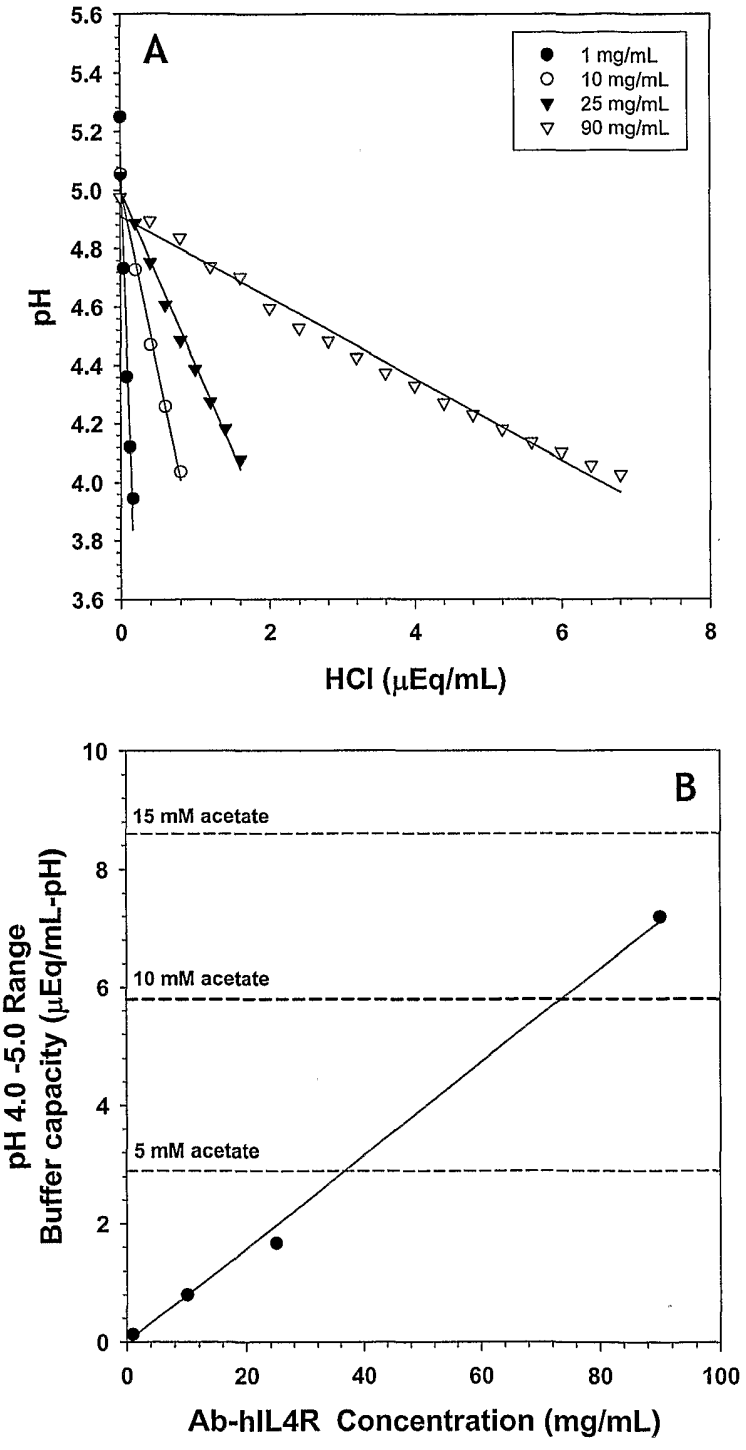


Figure 16. Buffer capacity of Ab-hIL4R Solutions in the pH 5.0 - 6.0 Range

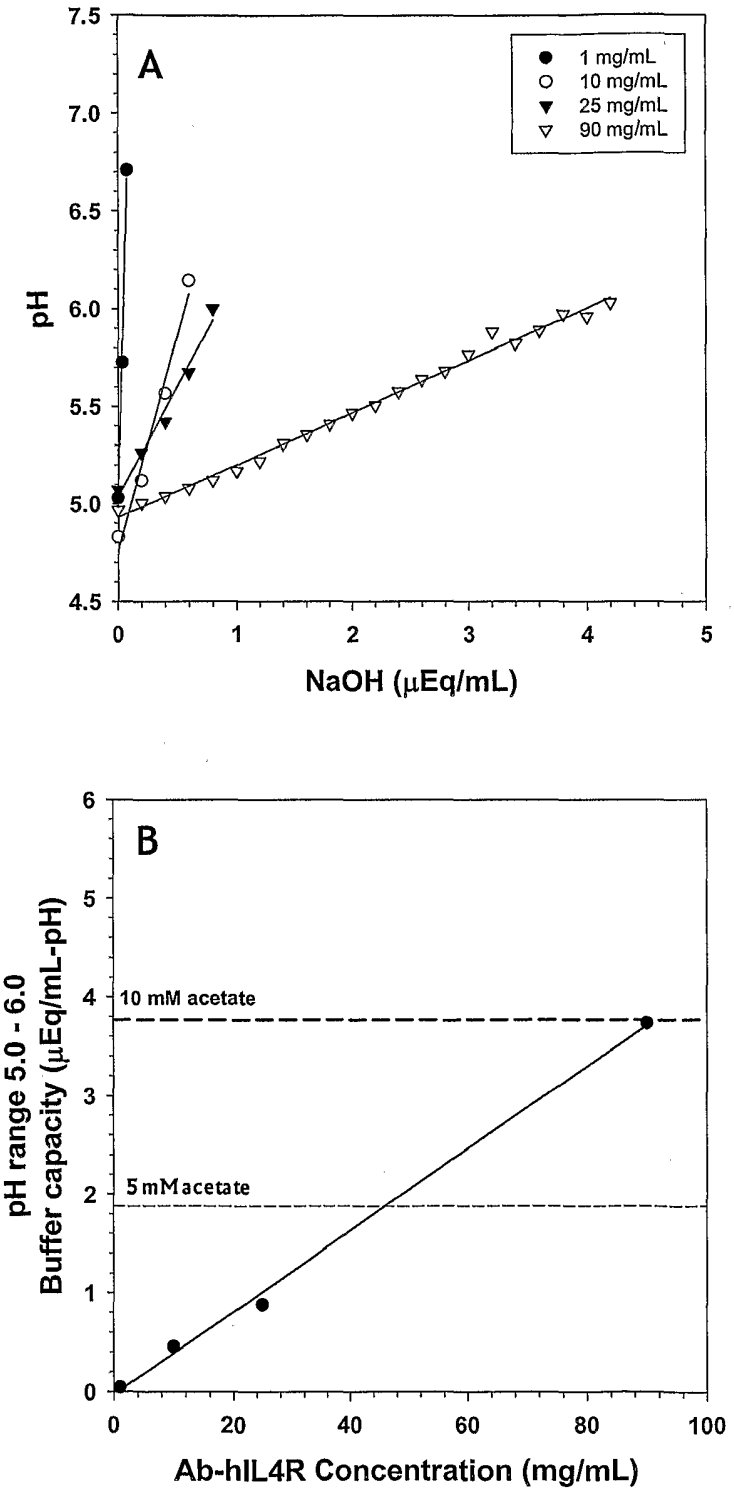


Figure 17. Accelerated Stability & pH control of the Self-Buffered, 70 mg/mL, Ab-hIL4R Formulation Compared to the Acetate Buffered Formulation at 37 C

