COMPOUNDED MEDIA POWDER FORMULATION AND METHOD OF PREPARATION OF LIQUID MEDIUM FOR CELL CULTURE

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

Incorporated by reference in its entirety herein is a computer-readable sequence listing submitted concurrently herewith and identified as follows: One (66,989 Byte ASCII (Text)) file named "sequence_listing_ST25.txt," created on April 10, 2014.

FIELD

The invention relates to the fields of cell biology and cell culture.

BACKGROUND

Various media for growing eukaryotic cells in culture are described in the literature and are commercially available. Cell culture media functions to provide the cells with a suitable pH and osmolality and nutrients essential for cell survival, growth and protein expression.

Examples of some common basal culture media are RPMI Media 1640, Medium 199, Minimal Essential Medium (MEM) medium (a "minimal" medium for growth of attached mammalian cells), Leibovitz medium for growth in absence of CO_2, Dulbecco's Modified Eagle's Medium and Ham's F12 Medium. The various media are distinguished from one another in that they contain different components in precise amino acids, vitamins, organic salts, trace elements, and other organic compounds which promote the growth of (and protein expression by) the cultured cells.

The development of an optimal cell culture medium is of significant importance, because changes to various components can lead to unexpected improvements in cell growth, increased growth rates, growth to high cell densities, improvements in controlling the stage and amount of cell differentiation, increased protein secretion, increased phenotypic and genetic stability, and elimination of

senescence for many cell types, all of which are consequential properties when producing recombinant proteins on a commercial scale.

Preparation of cell culture media, in particular in a commercial setting, is complex, and usually requires the stocking, transfer, preparation and storage of multiple stock solutions and powders and batching in a sequential manner. The large number of process steps and components in media preparation reduce efficiency and increase costs; they can also introduce variability, which in turn can impact the growth of cells and protein production. Hydration is also a significant obstacle to overcome, because as more and more components are added to a media formulation, the more difficult and less predictable it becomes to completely hydrate and dissolve the components to achieve a homogeneous mixture and an optimal osmolality, due to the richness and complexity of the composition, and interactions amongst the various components. For this reason, some components are often hydrated separately in complex media formulations. However, this results in increased cost and complexity during production.

Accordingly, there remains a need in the art for improved/further compounded dry media powder (DMP) formulations and methods of making the same in order to reduce complexity and lower costs while maintaining the correct composition in the fully hydrated liquid media as well as similar cell culture performance and recombinant protein production.

SUMMARY

In one aspect, the invention provides a compounded cell culture medium powder formulation comprising: a basal medium powder and a cell culture media supplement, wherein the cell culture media supplement comprises: one or more salts; one or more growth factors; one or more inorganic ions; an amino acid supplement comprising one or more of asparagine, glutamine, histidine, and serine; one or more buffers; and one or more anti-foaming agents.

In some embodiments, the basal medium powder comprises Dulbecco's Modified Eagle's Medium, Ham's Medium F12, Eagle's Minimal Essential

Medium, RPMI 1640 Medium, Dulbecco's Modified Eagle's Medium/Ham's F12 Medium (DMEM/F-12; 1:1 ratio), and combinations or modifications thereof.

In some embodiments, the basal medium powder comprises one or more of the following components or a combination thereof: biotin, calcium chloride, choline chloride, cyanocobalamin (B12), D+ mannose, D-calcium pantothenate, dextrose (anhydrous), DL-alpha-lipoic acid, ferric nitrate $9H_20$, ferrous sulfate $7H_20$, folic acid, glycine, hypoxanthine, I-inositol, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine HCl H_20, L-cystine 2HCl, L-glutamic acid (anhydrous), L-glutamine, L-glutathione, L-histidine FB, L-histidine HCl, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylaline, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, magnesium chloride, magnesium sulfate anhydrous, niacinamide, O-phosphoryl-ethanolamine, potassium chloride, putrescine 2HCl, pyridoxal HCl, pyridoxine HCl, riboflavin, sodium chloride, sodium phosphate monobasic H_20, sodium phosphate dibasic anhydrous, sodium pyruvate, thiamine HCl, thymidine, zinc sulfate $7H_20$, cupric sulfate, selenium dioxide, linoleic acid, beta-mercaptoethanol and ethanolamine free-base FB.

In some embodiments, the basal medium powder has a concentration of 8-30 g/L when the compounded cell culture medium powder formulation is combined with water to form a cell culture medium. In some embodiments, the basal medium powder has a concentration of 12-14 g/L when the compounded cell culture medium powder formulation is combined with water to form a cell culture medium. In some embodiments, the basal medium powder has a concentration of about 13 g/L when the compounded cell culture medium powder formulation is combined with water to form a cell culture medium.

In some embodiments, the compounded cell culture medium powder formulation further comprises a pH indicator. In some embodiments, the pH indicator is Phenol Red Na, wherein the Phenol Red Na is present at a concentration of about 0.001 to about 0.02 g/L when the compounded cell culture medium powder formulation is combined with water to form a cell culture medium. In some embodiments, the Phenol Red Na is present at a concentration of about 0.0069 g/L

when the compounded cell culture medium powder formulation is combined with water to form a cell culture medium.

In some embodiments, the basal medium powder components provide the following final concentration upon hydration to form a cell culture medium:

i) 0.0003-0.003 g/L biotin;

ii) 0.035-0.33 g/L calcium chloride;

iii) 0.003-0.03 g/L choline chloride;

iv) 0.0002-0.002 g/L cyanocobalamin (B12);

v) 1-10 g/L D+ mannose;

vi) 0.001-0.01 g/L D-calcium pantothenate;

vii) 0.3-3.0 g/L dextrose (anhydrous);

viii) 0.00003-0.0003 g/L DL-alpha-lipoic acid;

ix) 0.00002-0.00015 g/L ferric nitrate $9H_20$;

x) 0.0001-0.0015 g/L ferrous sulfate $7H_20$;

xi) 0.001-0.01 g/L folic acid;

xii) 0.007-0.20 g/L glycine;

xiii) 0.001-0.01 g/L hypoxanthine 2Na;

xiv) 0.005-0.05 g/L I-inositol;

xv) 0.003-0.03 g/L L-alanine;

xvi) 0.08-1.4 g/L L-arginine;

xvii) 0.006-0.16 g/L L-asparagine;

xviii) 0.005-0.10 g/L L-aspartic acid;

xix) 0.005-0.05 g/L L-cysteine HCl H_20;

xx) 0.02-0.2 g/L L-cystine 2HCl;

xxi) 0.005-0.15 g/L L-glutamic acid (anhydrous);

xxii) 0.02-1.5 g/L L-glutamine;

xxiii) 0.0003-0.003 g/L L-glutathione;

xxiv) 0.02-0.2 g/L L-histidine HCl;

xxv) 0.03-0.9 g/L L-isoleucine;

xxvi) 0.03-0.9 g/L L-leucine;

xxvii) 0.05-1.5 g/L L-lysine;

xxviii) 0.01-0.3 g/L L-methionine;

xxix) 0.02-0.6 g/L L-phenylaline;

xxx) 0.008-0.25 g/L L-proline;

xxxi) 0.009-0.25 g/L L-serine;

xxxii) 0.03-0.9 g/L L-threonine;

xxxiii) 0.006-0.16 g/L L-tryptophan;

xxxiv) 0.03-0.9 g/L L-tyrosine 2Na $2H_2O$;

xxxv) 0.03-0.9 g/L L-valine;

xxxvi) 0.01-0.18 g/L magnesium chloride;

xxxvii) 0.02-0.12 g/L magnesium sulfate anhydrous;

xxxviii) 0.001-0.01 g/L niacinamide;

xxxix) 0.0005-0.005 g/L O-phoshphoryl-ethanolamine;

xl) 0.1-1.0 g/L potassium chloride;

xli) 0.00002-0.0002 g/L putrescine 2HCl;

xlii) 0.001-0.01 g/L pyridoxal HCl;

xliii) 0.00001-0.0001 g/L pyridoxine HCl,

xliv) 0.0001-0.001 g/L riboflavin,

xlv) 2.0-15 g/L sodium chloride,

xlvi) 0.02-0.2 g/L sodium phosphate monobasic H_20,

xlvii) 0.02-0.2 g/L sodium phosphate dibasic anhydrous,

xlviii) 0.015-0.15 g/L sodium pyruvate,

xlix) 0.001-0.01 g/L thiamine HCl,

l) 0.0001-0.001 g/L thymidine,

li) 0.00015-0.0015 g/L zinc sulfate $7H_20$,

lii) 0.0000006-0.000006 g/L cupric sulfate $5H_2O$,

liii) 0.0000005-0.000008 g/L selenium dioxide,

liv) 0.00001-0.0001 g/L linoleic acid,

lv) 0.0001-0.001 g/L beta-mercaptoethanol; and

lvi) 0.0003-0.005 g/L ethanolamine FB.

In some embodiments, the basal medium powder components provide the following final concentration upon hydration to form a cell culture medium:

i) about 0.001 g/L biotin;

ii) about 0.11665 g/L calcium chloride;

iii) about 0.00998 g/L choline chloride;

iv) about 0.00068 g/L cyanocobalamin (B12);

v) about 3 g/L D+ mannose;

vi) about 0.00312 g/L D-calcium pantothenate;

vii) about 1 g/L dextrose (anhydrous);

viii) about 0.000103 g/L DL-alpha-lipoic acid;

ix) about 0.00005 g/L ferric nitrate $9H_20$;

x) about 0.000417 g/L ferrous sulfate $7H_20$;

xi) about 0.00366 g/L folic acid;

xii) about 0.02626 g/L glycine;

xiii) about 0.0027 g/L hypoxanthine 2Na;

xiv) about 0.01451 g/L I-inositol;

xv) about 0.01336 g/L L-alanine;

xvi) about 0.27435 g/L L-arginine;

xvii) about 0.0225 g/L L-asparagine;

xviii) about 0.01995 g/L L-aspartic acid;

xix) about 0.01756 g/L L-cysteine HCl H_20;

xx) about 0.06256 g/L L-cystine 2HCl;

xxi) about 0.02206 g/L L-glutamic acid (anhydrous);

xxii) about 0.73 g/L L-glutamine;

xxiii) about 0.001 g/L L-glutathione;

xxiv) about 0.07348 g/L L-histidine HCl;

xxv) about 0.1057 g/L L-isoleucine;

xxvi) about 0.11096 g/L L-leucine;

xxvii) about 0.16385 g/L L-lysine;

xxviii) about 0.03224 g/L L-methionine;

xxix) about 0.06748 g/L L-phenylaline;

xxx) about 0.02875 g/L L-proline;

xxxi) about 0.03676 g/L L-serine;

xxxii) about 0.10156 g/L L-threonine;

xxxiii) about 0.01902 g/L L-tryptophan;

xxxiv) about 0.10771 g/L L-tyrosine 2Na $2H_2O$;

xxxv) about 0.09866 g/L L-valine;

xxxvi) about 0.028 g/L magnesium chloride;

xxxvii) about 0.04884 g/L magnesium sulfate anhydrous;

xxxviii) about 0.00302 g/L niacinamide;

xxxix) about 0.0014 g/L O-phoshphoryl-ethanolamine;

xl) about 0.31182 g/L potassium chloride;

xli) about 0.000081 g/L putrescine 2HCl;

xlii) about 0.003 g/L pyridoxal HCl;

xliii) about 0.000031 g/L pyridoxine HCl,

xliv) about 0.000319 g/L riboflavin,

xlv) about 6.1234 g/L sodium chloride,

xlvi) about 0.0625 g/L sodium phosphate monobasic H_20,

xlvii) about 0.07099 g/L sodium phosphate dibasic anhydrous,

xlviii) about 0.055 sodium pyruvate,

xlix) about 0.00317 g/L thiamine HCl,

l) about 0.000364 g/L thymidine,

li) about 0.000432 g/L zinc sulfate $7H_20$,

lii) about 0.00000125 g/L cupric sulfate $5H_2O$,

liii) about 0.00000222 g/L selenium dioxide,

liv) about 0.000042 g/L linoleic acid,

lv) about 0.00039065 g/L beta-mercaptoethanol; and

lvi) about 0.0012 g/L ethanolamine FB.

In some embodiments, the one or more salts of the cell culture media supplement comprises magnesium chloride in a concentration of 0.5-5 g/L upon hydration to form a cell culture medium. In some embodiments, the magnesium chloride has a concentration of about 1.428 g/L upon hydration to form a cell culture medium.

In some embodiments, the cell culture media supplement comprises recombinant insulin as a growth factor. In some embodiments, the insulin has a concentration of 0.5-15 mg/L upon hydration to form a cell culture medium. In

some embodiments, the insulin has a concentration of about 3 mg/L upon hydration to form a cell culture medium.

In some embodiments, the one or more inorganic ions of the cell culture media supplement comprises trace metals selected ammonium molybdate, chromium potassium sulfate, cupric sulfate, lithium chloride, manganese sulfate, sodium metasilicate and combinations thereof. In some embodiments, the ammonium molybdate is ammonium molybdate $4H_2O$; the chromium potassium sulfate is chromium potassium sulfate $12H_2O$, the cupric sulfate is cupric sulfate $5H_2O$, the lithium chloride is lithium chloride (anhydrous), the manganese sulfate is manganese sulfate H_2O and the sodium metasilicate is sodium metasilicate $9H_2O$.

In some embodiments, the trace metals provide the following final concentration upon hydration to form a cell culture medium:

i) 0.0005-0.01 mg/L of ammonium molybdate $4H_2O$;

ii) 0.0001-0.01 mg/L of chromium potassium sulfate $12H_2O$;

iii) 0.001-0.125 mg/L of cupric sulfate $5H_2O$;

iv) 0.001-0.1 mg/L of lithium chloride (anhydrous);

v) 0.00004-0.004 mg/L of manganese sulfate H_2O; and

vi) 0.04-4.2 mg/L of sodium metasilicate $9H_2O$.

In some embodiments, the cell culture media supplement comprises a combination of ammonium molybdate $4H_2O$, chromium potassium sulfate $12H_2O$, cupric sulfate $5H_2O$, lithium chloride (anhydrous), manganese sulfate H_2O, and sodium metasilicate 9H2O. In some embodiments, the trace metals provide the following final concentration upon hydration to form a cell culture medium:

i) about 0.0037 mg/L of ammonium molybdate $4H_2O$;

ii) about 0.001 mg/L of chromium potassium sulfate $12H_2O$;

iii) about 0.0125 mg/L of cupric sulfate $5H_2O$;

iv) about 0.01 mg/L of lithium chloride (anhydrous);

v) about 0.000452 mg/L of manganese sulfate H_2O; and

vi) about 0.4263 mg/L of sodium metasilicate $9H_2O$.

In some embodiments, the amino acid supplement of the cell culture media supplement comprises a combination of asparagine H_2O, glutamine, histidine, and serine. In some embodiments, the amino acid supplement has the following final concentration upon hydration to form a cell culture medium:

i) 0.007-0.07 g/L asparagine H_2O;

ii) 0.25-2.5 g/L glutamine;

iii) 0.5-5.0 g/L histidine, free base; and

iv) 0.01-0.1 g/L serine.

In some embodiments, the amino acid supplement has the following final concentration upon hydration to form a cell culture medium:

i) about 0.0225 g/L asparagine H_2O;

ii) about 0.73 g/L glutamine;

iii) about 1.552 g/L histidine; and

iv) about 0.03676 g/L serine.

In some embodiments, the one or more buffers of the cell culture media supplement is selected from the group consisting of 3-(N-morpholino)propanesulfonic acid (MOPS) free acid, 3-(N-morpholino)propanesulfonic acid (MOPS) Na, hydroxyethyl piperazineethanesulfonic acid (HEPES) and sodium bicarbonate. In some embodiments, the formulation comprises a combination of MOPS free acid and MOPS Na. In some embodiments, the buffers provide the following final concentration upon hydration to form a cell culture medium:

i) 0.3-3 g/L MOPS free acid; and

ii) 1.0-10 g/L MOPS Na.

In some embodiments, the anti-foaming agent of the cell culture media supplement comprises a polyol copolymer based on ethylene oxide and propylene oxide. In some embodiments, the anti-foaming agent is Pluronic F68. In some

embodiments, the Pluronic F68 has a concentration of 0.1-10 g/L upon hydration to form a cell culture medium. In some embodiments, the Pluronic F68 has a concentration of about 1 g/L upon hydration to form a cell culture medium.

In another aspect, the invention provides a method of making the compounded cell culture medium powder formulation of the invention, comprising combining the basal medium powder; one or more salts; one or more growth factors; one or more inorganic ions; an amino acid supplement comprising one or more of asparagine, glutamine, histidine, and serine; one or more buffers; and one or more anti-foaming agents.

In another aspect, the invention provides a method of making a cell culture medium for growing mammalian cells, comprising contacting the compounded cell culture medium powder formulation with water, thereby making a cell culture medium for growing mammalian cells. In some embodiments, the components are substantially dissolved in the water. In some embodiments, the method further comprises combining a solution comprising $FeSO_4$ $7H_2O$ and a chelating agent. In some embodiments, the chelating agent is ethylenediaminetetraacetic acid (EDTA). In some embodiments, the $FeSO_4$ $7H_2O$ and EDTA have the following final concentration in the cell culture medium: 0.004-0.04 g/L $FeSO_4$ $7H_2O$; and 0.006-0.06 g/L EDTA. In some embodiments, the $FeSO_4$ $7H_2O$ and EDTA have the following final concentration in the cell culture medium: about 0.0138g/L $FeSO_4$ $7H_2O$; and about 0.018625 g/L EDTA.

In another embodiment, the method further comprises contacting the cell culture medium of the invention with cells. In some embodiments, the cells are mammalian cells.

In another embodiment, the invention provides a method of culturing cells, comprising, comprising contacting cells with a cell culture medium of the invention and culturing the cells in the medium for a period of time.

In another embodiment, the invention provides a method of producing a protein of interest, comprising contacting cells expressing the protein of interest with a cell culture medium; culturing the cells in the medium for a period of time; and isolating the protein of interest from the cell culture medium.

In some embodiments, the mammalian cells are selected from the group consisting of Chinese Hamster Ovary (CHO) cells, DP12 CHO cells, DG44 CHO cells, Human Embryonic Kidney (HEK) cells, HEK 293 cells, and baby hamster kidney (BHK) cells. In some embodiments, the mammalian cells recombinantly express a protein of interest. In some embodiments, the protein of interest is selected from the group consisting of coagulation Factor VIII (FVIII), variants and fragments thereof. In some embodiments, the FVIII is selected from wild-type FVIII, B-domain deleted FVIII and FVIII conjugated with a biocompatible polymer. In some embodiments, the biocompatible polymer is polyethylene glycol (PEG). In some embodiments, the PEG is covalently attached to the polypeptide at one or more of the factor VIII amino acid positions 81, 129, 377, 378, 468, 487, 491, 504, 556, 570, 711, 1648, 1795, 1796, 1803, 1804, 1808, 1810, 1864, 1903, 1911, 2091, 2118 and 2284.

These and other features of the present teachings are set forth herein.

BRIEF DESCRIPTION OF THE FIGURES

The skilled artisan will understand that the drawings, described below, are for illustration purposes only. The drawings are not intended to limit the scope of the present teachings in any way.

FIG. 1. Illustration of non-compounded media preparation. Most (e.g., 57) medium components are contained in the Base Media powder, which can be premixed. Additional media components are added separately to their respective target final concentration to complete the formulation. $FeSO_4$ / EDTA, Trace Metals Panel, and rH insulin are prepared as stock solutions and added to media batches as liquids while magnesium chloride and the supplement are added as powders. The complete medium contains basic cell nutrients, salts, carbohydrates, anti-shear factors, amino acids, vitamins, trace metals, insulin and various other chemicals that aid in cell growth and recombinant protein production and stabilize the recombinant protein product secreted from the cell.

FIG. 2. Illustration of cell culture media preparation using a (nearly fully-compounded) termed herein as “compounded” formulation. Ferrous sulfate/EDTA is not capable of compounding, apparently due to unintended chelation of other

bivalent trace metals (existing in the medium) by the EDTA and is therefore added separately.

FIG. 3. Cell culture runs using current and compounded method of preparation demonstrated similar growth properties, metabolism and product titer (measured as Potency). Media was prepared the standard way using base media powder (v.1) and sequential supplements addition (Fig. 1) and using compounded media powder (v3.3) with only ferrous sulfate/EDTA addition (Fig. 2) and used to cultivate rFVIII-expressing cells in 1L perfusion bioreactor runs. No change was apparent in cell culture performance and rFVIII titer (potency) after shifting from non-compounded to compounded medium.

DETAILED DESCRIPTION

The present inventor has developed a compounded cell culture medium powder formulation useful for making a cell culture medium and for recombinant protein production. Described herein are formulations of compounded mammalian cell culture medium powder, methods of making the same and a hydration method which can reduce cost, simplify workflow, and reduce complexity of medium preparation – all which are especially important in commercial settings, among other advantages described herein.

For the purpose of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth below conflicts with the usage of that word in any other document, including any document incorporated herein by reference, the definition set forth below shall always control for purposes of interpreting this specification and its associated claims unless a contrary meaning is clearly intended (for example in the document where the term is originally used). The use of "or" means "and/or" unless stated otherwise. The use of “a” herein means “one or more” unless stated otherwise or where the use of “one or more” is clearly inappropriate. The use of “comprise,” “comprises,” “comprising,” “include,” “includes,” and “including” are interchangeable and not intended to be limiting. Furthermore, where the description of one or more embodiments uses the term “comprising,” those skilled in the art would understand that, in some specific

instances, the embodiment or embodiments can be alternatively described using the language "consisting essentially of" and/or "consisting of."

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which this invention pertains. The following references provide one of skill with a general definition of many of the terms used in this invention: *Academic Press Dictionary of Science and Technology*, Morris (Ed.), Academic Press (1st ed., 1992); *Oxford Dictionary of Biochemistry and Molecular Biology*, Smith et al. (Eds.), Oxford University Press (revised ed., 2000); *Encyclopaedic Dictionary of Chemistry*, Kumar (Ed.), Anmol Publications Pvt. Ltd. (2002); *Dictionary of Microbiology and Molecular Biology*, Singleton et al. (Eds.), John Wiley & Sons (3rd ed., 2002); *Dictionary of Chemistry*, Hunt (Ed.), Routledge (1st ed., 1999); *Dictionary of Pharmaceutical Medicine*, Nahler (Ed.), Springer-Verlag Telos (1994); *Dictionary of Organic Chemistry*, Kumar and Anandand (Eds.), Anmol Publications Pvt. Ltd. (2002); and *A Dictionary of Biology (Oxford Paperback Reference)*, Martin and Hine (Eds.), Oxford University Press (4th ed., 2000). Practitioners are also directed to Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual (Second Edition)*, Cold Spring Harbor Press, Plainview, N.Y.; Ausubel F M *et al.* (1993) and *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, N.Y.; and Gelvin and Schilperoot, eds. (1997), for definitions and terms of the art. Further clarifications of some of these terms as they apply specifically to this invention are provided herein.

The term "about" with respect to the compositions means plus or minus a range of up to 20%.

As used herein, the terms "cell," "cells," "cell line," "host cell," and "host cells," are used interchangeably and, encompass plant and animal cells and include invertebrate, non-mammalian vertebrate and mammalian cells. All such designations include cell populations and progeny. Thus, the terms "transformants" and "transfectants" include the primary subject cell and cell lines derived therefrom without regard for the number of transfers. Exemplary non-mammalian vertebrate cells include, for example, avian cells, reptilian cells and amphibian cells. Exemplary invertebrate cells include, but are not limited to, insect cells such as, for

example, caterpillar (*Spodoptera frugiperda*) cells, mosquito (*Aedes aegypti*) cells, fruitfly (*Drosophila melanogaster*) cells, Schneider cells, and Bombyx mori cells. *See*, *e.g.*, Luckow *et al.*, *Bio/Technology* 6:47-55 (1988). The cells may be differentiated, partially differentiated or undifferentiated, e.g. stem cells, including embryonic stem cells and pluripotent stem cells. Additionally tissue samples derived from organs or organ systems may be used according to the invention.

The terms "cell culture," or "tissue culture" refer to cells grown in suspension or grown adhered to a variety of surfaces or substrates in vessels such as roller bottles, tissue culture flasks, dishes, multi-well plates and the like. Large scale approaches, such as bioreactors, including adherent cells growing attached to microcarriers in stirred fermentors, are also encompassed by the term "cell culture." Moreover, it is possible not only to culture contact-dependent cells, but also to use suspension culture techniques in the methods of the claimed invention. Exemplary microcarriers include, for example, dextran, collagen, plastic, gelatin and cellulose and others as described in Butler, Spier & Griffiths, *Animal cell Biotechnology* 3:283-303 (1988). Porous carriers, such as, for example, Cytoline™ or Cytopore™, as well as dextran-based carriers, such as DEAE-dextran (Cytodex 1™ quaternary amine-coated dextran (Cytodex™) or gelatin-based carriers, such as gelatin-coated dextran (Cytodex 3™) may also be used. Cell culture procedures for both large and small-scale production of proteins are encompassed by the present invention. Procedures including, but not limited to, a fluidized bed bioreactor, hollow fiber bioreactor, roller bottle culture, or stirred tank bioreactor system may be used, with or without microcarriers, and operated alternatively in a batch, fed-batch, or perfusion mode.

The term "mammalian host cell," "mammalian cell," "mammalian recombinant host cell," and the like, refer to cell lines derived from mammals that are capable of growth and survival when placed in either monolayer culture or in suspension culture in a medium containing the appropriate nutrients and growth factors. Exemplary mammalian cells include, for example, cells derived from human, non-human primate, cat, dog, sheep, goat, cow, horse, pig, rabbit, rodents including mouse, hamster, rat and guinea pig and any derivatives and progenies thereof. Typically, the cells are capable of expressing and secreting large quantities

of a particular protein of interest (typically a recombinant protein) into the culture medium, and are cultured for this purpose. However, the cells may be cultured for a variety of other purposes as well, and the scope of this invention is not limited to culturing the cells only for production of recombinant proteins. Examples of suitable mammalian cell lines, capable of growth in the media of this invention, include monkey kidney CVI line transformed by SV40 (COS-7, ATCC® CRL 1651); human embryonic kidney line 293S (Graham *et al.*, *J. Gen. Virolo.*, 36:59 (1977)); baby hamster kidney cells (BHK, ATCC® CCL 10); mouse sertoli cells (TM4, Mather, *Biol. Reprod.*, 23:243 (1980)); monkey kidney cells (CVI-76, ATCC® CCL 70); African green monkey kidney cells (VERO-76, ATCC® CRL-1587); human cervical carcinoma cells (HELA, ATCC® CCL 2); canine kidney cells (MDCK, ATCC® CCL 34); buffalo rat liver cells (BRL 3A, ATCC® CRL 1442); human lung cells (W138, ATCC® CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor cells (MMT 060562, ATCC® CCL SI); rat hepatoma cells (HTC, MI.54, Baumann *et al.*, *J. Cell Biol.*, 85:1 (1980)); and TR-1 cells (Mather *et al.*, *Annals N.Y. Acad. Sci.*, 383:44 (1982)) and hybridoma cell lines. In some embodiments, Chinese hamster ovary cells (Urlab and Chasin, *Proc. Natl. Acad. Sci.* USA, 77:4216 (1980)) can be grown in the media. CHO cells suitable for use in the methods of the present invention have also been described in the following documents: EP 117,159, published Aug. 29, 1989; U.S. Pat. Nos. 4,766,075; 4,853,330; 5,185,259; Lubiniecki *et al.*, in *Advances in Animal Cell Biology and Technology for Bioprocesses*, Spier *et al.*, eds. (1989), pp. 442-451. Known CHO derivatives suitable for use herein include, for example, CHO/-DHFR (Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77: 4216 (1980)), CHO-K1 DUX B11 (Simonsen and Levinson, *Proc. Natl. Acad. Sci. USA* 80: 2495-2499 (1983); Urlaub and Chasin, *supra*), and dp 12.CHO cells (EP 307,247 published Mar. 15, 1989). In one embodiment, the cells useful for growth in the media are selected form Chinese Hamster Ovary (CHO) cells, DP12 CHO cells, DG44 CHO cells, Human Embryonic Kidney (HEK) cells, HEK 293 cells, and baby hamster kidney (BHK) cells. In some embodiments, the cells express a protein or proteins of interest. In some embodiments, the cells have been engineered to recombinantly express the protein.

In one embodiment, the invention provides a compounded cell culture medium powder formulation comprising a basal medium powder and a cell culture media supplement, wherein the cell culture media supplement comprises one or more salts; one or more growth factors; one or more inorganic ions; an amino acid supplement comprising one or more of asparagine, glutamine, histidine, and serine; one or more buffers; and one or more anti-foaming agents.

The compounded cell culture medium powder formulation is generally for use as a cell culture medium which is "serum free" wherein the medium is essentially free of serum from any mammalian source (e.g. fetal bovine serum (FBS)). By "essentially free" is meant that the cell culture medium comprises between about 0-5% serum, preferably between about 0-1% serum, and most preferably between about 0-0.1% serum. Advantageously, serum-free "defined" medium can be used, wherein the identity and concentration of each of the components in the medium is known (i.e., an undefined component such as bovine pituitary extract (BPE) is not present in the culture medium).

Notwithstanding, the compounded cell culture dry powder medium described in the present invention can also be used in formulations where serum from various sources and/or animals and/or combinations or derivatives thereof (e.g., FBS or human plasma protein-fraction solution, HPPS, and/or the like), is/are added to generate the final formulation for cultivating cells.

The term "basal medium powder" or "basal media" refers to cell culture media that may contain, for example, any or all of the following components: proteins, lipids, carbohydrates, amino acids, organic and/or inorganic salts, buffers (e.g., bicarbonate), vitamins, hormones, antibiotics, and pH indicators (e.g., phenol red). Examples of cell culture media bases that can be used in accordance with the present invention are not limiting and can include: Dulbecco's Modified Eagle's Medium (DMEM), Ham's F12 Medium, MCDB Media, Minimum Essential Medium Eagle, RPMI Media, Ames' Media, BGJb Medium (Fitton-Jackson Modification), Click's Medium, CMRL-1066 Medium, Fischer's Medium, Glascow Minimum Essential Medium (GMEM), Iscove's Modified Dulbecco's Medium (IMDM), L-15 Medium (Leibovitz), McCoy's 5A Modified Medium, NCTC Medium, Swim's S-77 Medium, Waymouth Medium, and William's Medium E. In

some embodiments, the basal medium powder is a combination or a modification of the above listed cell culture media bases. In some embodiments, the basal media powder is Dulbecco's Modified Eagle's Medium/Ham's F12 Medium (DMEM/F-12; 1:1 ratio).

In some embodiments, the basal medium powder comprises one or more of the following components or a combination thereof: biotin, calcium chloride, choline chloride, cyanocobalamin (B12), D+ mannose, D-calcium pantothenate, dextrose (anhydrous), DL-alpha-lipoic acid, ferric nitrate $9H_20$, ferrous sulfate $7H_20$, folic acid, glycine, hypoxanthine, I-inositol, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine HCl H_20, L-cystine 2HCl, L-glutamic acid (anhydrous), L-glutamine, L-glutathione, L-histidine FB, L-histidine HCl, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylaline, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, magnesium chloride, magnesium sulfate anhydrous, niacinamide, O-phosphoryl-ethanolamine, potassium chloride, putrescine 2HCl, pyridoxal HCl, pyridoxine HCl, riboflavin, sodium chloride, sodium phosphate monobasic H_20, sodium phosphate dibasic anhydrous, sodium pyruvate, thiamine HCl, thymidine, zinc sulfate $7H_20$, cupric sulfate, selenium dioxide, linoleic acid, beta-mercaptoethanol and ethanolamine free-base FB.

Cell culture media components are generally available from the following sources: Research Organics Inc., United Biochemicals, Angus Chemical Company, (Mikrochem) Bayer Biotechnology, Kyowa Hakko U.S.A. Inc., Ferro/Pfanstiehl, Sigma-Aldrich Inc., Research Organics Inc., VWR Scientific Inc., Ajinomoto AminoScience LLC, EMD MILLIPORE CORPORATION, Kyowa Hakko U.S.A. Inc., and Tilley Chemical Co., Inc.

In some embodiments, the compounded cell culture medium powder formulation comprises a pH indicator. The pH indicator is not limiting, provided that it is suitable for cell culture. In some embodiments, the pH indicator is Phenol Red Na. In some embodiments, Phenol Red Na is present at a concentration of about 0.001 to about 0.02 g/L when the compounded cell culture medium powder formulation is combined with water to form a cell culture medium. In some embodiments, the Phenol Red Na is present at a concentration of about 0.0069 g/L.

In some embodiments, the basal medium powder comprises the following components which provide the following final concentration upon hydration to form a cell culture medium:

i) 0.0003-0.003 g/L biotin;

ii) 0.035-0.33 g/L calcium chloride;

iii) 0.003-0.03 g/L choline chloride;

iv) 0.0002-0.002 g/L cyanocobalamin (B12);

v) 1-10 g/L D+ mannose;

vi) 0.001-0.01 g/L D-calcium pantothenate;

vii) 0.3-3.0 g/L dextrose (anhydrous);

viii) 0.00003-0.0003 g/L DL-alpha-lipoic acid;

ix) 0.00002-0.00015 g/L ferric nitrate $9H_20$;

x) 0.0001-0.0015 g/L ferrous sulfate $7H_20$;

xi) 0.001-0.01 g/L folic acid;

xii) 0.007-0.20 g/L glycine;

xiii) 0.001-0.01 g/L hypoxanthine 2Na;

xiv) 0.005-0.05 g/L I-inositol;

xv) 0.003-0.03 g/L L-alanine;

xvi) 0.08-1.4 g/L L-arginine;

xvii) 0.006-0.16 g/L L-asparagine;

xviii) 0.005-0.10 g/L L-aspartic acid;

xix) 0.005-0.05 g/L L-cysteine HCl H_20;

xx) 0.02-0.2 g/L L-cystine 2HCl;

xxi) 0.005-0.15 g/L L-glutamic acid (anhydrous);

xxii) 0.02-1.5 g/L L-glutamine;

xxiii) 0.0003-0.003 g/L L-glutathione;

xxiv) 0.02-0.2 g/L L-histidine HCl;

xxv) 0.03-0.9 g/L L-isoleucine;

xxvi) 0.03-0.9 g/L L-leucine;

xxvii) 0.05-1.5 g/L L-lysine;

xxviii) 0.01-0.3 g/L L-methionine;

xxix) 0.02-0.6 g/L L-phenylaline;

xxx) 0.008-0.25 g/L L-proline;

xxxi) 0.009-0.25 g/L L-serine;

xxxii) 0.03-0.9 g/L L-threonine;

xxxiii) 0.006-0.16 g/L L-tryptophan;

xxxiv) 0.03-0.9 g/L L-tyrosine 2Na $2H_2O$;

xxxv) 0.03-0.9 g/L L-valine;

xxxvi) 0.01-0.18 g/L magnesium chloride ;

xxxvii) 0.02-0.12 g/L magnesium sulfate anhydrous;

xxxviii) 0.001-0.01 g/L niacinamide;

xxxix) 0.0005-0.005 g/L O-phoshphoryl-ethanolamine;

xl) 0.1-1.0 g/L potassium chloride;

xli) 0.00002-0.0002 g/L putrescine 2HCl;

xlii) 0.001-0.01 g/L pyridoxal HCl;

xliii) 0.00001-0.0001 g/L pyridoxine HCl,

xliv) 0.0001-0.001 g/L riboflavin,

xlv) 2.0-15 g/L sodium chloride,

xlvi) 0.02-0.2 g/L sodium phosphate monobasic H_20,

xlvii) 0.02-0.2 g/L sodium phosphate dibasic anhydrous,

xlviii) 0.015-0.15 g/L sodium pyruvate,

xlix) 0.001-0.01 g/L thiamine HCl,

l) 0.0001-0.001 g/L thymidine,

li) 0.00015-0.0015 g/L zinc sulfate $7H_20$,

lii) 0.0000006-0.000006 g/L cupric sulfate $5H_2O$,

liii) 0.0000005-0.000008 g/L selenium dioxide,

liv) 0.00001-0.0001 g/L linoleic acid,

lv) 0.0001-0.001 g/L beta-mercaptoethanol; and

lvi) 0.0003-0.005 g/L ethanolamine FB.

In some embodiments, the basal medium powder comprises the following components which provide the following final concentration upon hydration to form a cell culture medium:

i) about 0.001 g/L biotin;

ii) about 0.11665 g/L calcium chloride;

iii) about 0.00998 g/L choline chloride;

iv) about 0.00068 g/L cyanocobalamin (B12);

v) about 3 g/L D+ mannose;

vi) about 0.00312 g/L D-calcium pantothenate;

vii) about 1 g/L dextrose (anhydrous);

viii) about 0.000103 g/L DL-alpha-lipoic acid;

ix) about 0.00005 g/L ferric nitrate $9H_20$;

x) about 0.000417 g/L ferrous sulfate $7H_20$;

xi) about 0.00366 g/L folic acid;

xii) about 0.02626 g/L glycine;

xiii) about 0.0027 g/L hypoxanthine 2Na;

xiv) about 0.01451 g/L I-inositol;

xv) about 0.01336 g/L L-alanine;

xvi) about 0.27435 g/L L-arginine;

xvii) about 0.0225 g/L L-asparagine;

xviii) about 0.01995 g/L L-aspartic acid;

xix) about 0.01756 g/L L-cysteine HCl H_20;

xx) about 0.06256 g/L L-cystine 2HCl;

xxi) about 0.02206 g/L L-glutamic acid (anhydrous);

xxii) about 0.73 g/L L-glutamine;

xxiii) about 0.001 g/L L-glutathione;

xxiv) about 0.07348 g/L L-histidine HCl;

xxv) about 0.1057 g/L L-isoleucine;

xxvi) about 0.11096 g/L L-leucine;

xxvii) about 0.16385 g/L L-lysine;

xxviii) about 0.03224 g/L L-methionine;

xxix) about 0.06748 g/L L-phenylaline;

xxx) about 0.02875 g/L L-proline;

xxxi) about 0.03676 g/L L-serine;

xxxii) about 0.10156 g/L L-threonine;

xxxiii) about 0.01902 g/L L-tryptophan;

xxxiv) about 0.10771 g/L L-tyrosine 2Na $2H_2O$;

xxxv) about 0.09866 g/L L-valine;

xxxvi) about 0.028 g/L magnesium chloride;

xxxvii) about 0.04884 g/L magnesium sulfate anhydrous;

xxxviii) about 0.00302 g/L niacinamide;

xxxix) about 0.0014 g/L O-phoshphoryl-ethanolamine;

xl) about 0.31182 g/L potassium chloride;

xli) about 0.000081 g/L putrescine 2HCl;

xlii) about 0.003 g/L pyridoxal HCl;

xliii) about 0.000031 g/L pyridoxine HCl,

xliv) about 0.000319 g/L riboflavin,

xlv) about 6.1234 g/L sodium chloride,

xlvi) about 0.0625 g/L sodium phosphate monobasic H_20,

xlvii) about 0.07099 g/L sodium phosphate dibasic anhydrous,

xlviii) about 0.055 sodium pyruvate,

xlix) about 0.00317 g/L thiamine HCl,

l) about 0.000364 g/L thymidine,

li) about 0.000432 g/L zinc sulfate $7H_20$,

lii) about 0.00000125 g/L cupric sulfate $5H_2O$,

liii) about 0.00000222 g/L selenium dioxide,

liv) about 0.000042 g/L linoleic acid,

lv) about 0.00039065 g/L beta-mercaptoethanol; and

lvi) about 0.0012 g/L ethanolamine FB.

The components that can be used in the compounded media formulation as discussed herein can be in an anhydrous or in a hydrated form, and many such anhydrous and hydrated forms of the components are known by persons skilled in the art. For example, as indicated above, in some embodiments, the composition comprises hydrated forms of cupric sulfate, zinc sulfate, sodium phosphate monobasic, L-tyrosine, L-cysteine, ferric nitrate, and ferrous sulfate. Each of these components can be substituted with anhydrous forms, and the concentration ranges

can be recalculated based on differences in molecular weight between the hydrated and anhydrous forms. Likewise, any anhydrous forms mentioned in the specification can be substituted with hydrated forms, and the concentration ranges recalculated accordingly.

In some embodiments, the basal medium powder has a concentration of 8-30 g/L upon hydration to form a cell culture medium. In some embodiments, the basal medium powder has a concentration of 12-14 g/L upon hydration to form a cell culture medium. In some embodiments, the basal medium powder has a concentration of about 13 g/L upon hydration to form a cell culture medium.

The one or more salts of the cell culture media supplement is not limiting and includes any salts, and hydration state thereof, which are suitable for use in cell culture. In some embodiments, the one or more salts is selected from NaCl, KCl, NaH_2PO_4, $NaHCO_3$, $CaCl_2$, and $MgCl_2$ and combinations thereof. In some embodiments, the amount of salt in the supplement has a concentration of 0.5-5 g/L upon hydration to form a cell culture medium. In one embodiment, the salt of the cell culture media supplement is magnesium chloride. In some embodiments, the magnesium chloride of the supplement has a concentration of about 1.428 g/L upon hydration to form a cell culture medium.

The one or more growth factors of the cell culture media supplement are not limiting. In some embodiments, the growth factor is Amphiregulin, Angiopoietin, Betacellulin, (Bone Morphogenic protein-13, Bone Morphogenic protein-14, Bone Morphogenic protein-2, Human BMP-3, Bone Morphogenic protein-4, Human BMP-5, Bone Morphogenic protein-6, Bone Morphogenic protein-7, Human CD135 Ligand/Flt-3 Ligand, Human Granulocyte Colony Stimulating Factor (G-CSF), Human Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), Human Macrophage Colony Stimulating Factor (M-CSF), Human Cripto-1, Human CTGF (Connective tissue growth factor), Human EGF (Epidermal Growth Factor), Human EG-VEGF (Endocrine-Gland-Derived Vascular Endothelial Growth Factor), Human Erythropoietin (EPO), Human FGF (Fibroblast Growth Factors 1-23), Human GDF-11, Human GDF-15, Human GDF-8, Human Growth Hormone Releasing Factor (GHRF, GRF, GHRH, Growth Hormone Releasing Hormone), Human Heparin

Binding Epidermal Growth Factor (HB-EGF), Human Hepatocyte Growth Factor (HGF), Human Heregulin beta 1, Human insulin, Human IGF-1 (Insulin-like Growth Factor-1), Human IGF-2 (Insulin-like Growth Factor-2), Human IGFBP-1 (Insulin-like Growth Factor Binding Protein 1), Human IGFBP-3 (Insulin-like Growth Factor Binding Protein 3), intestinal trefoil factor (ITF), Human keratinocyte growth factors 1 & 2, Human Leukemia Inhibitory Factor (LIF), Human MSP, Human Myostatin, Human Myostatin, pro (propeptide), Human NRG1, Human NGF, Human Oncostatin M, Human Osteoblast Specific Factor 1 (OSF-1, Pleiotrophin), Human PD-ECGF (Platelet-derived endothelial cell growth factor), Human PDGF, Human PIGF, Human Placental Growth Factor 1 (PLGF1), Human Placental Growth Factor 2 (PLGF2), Human SCGF-a (Stem Cell Growth Factor-alpha), Human SCGF-b (Stem Cell Growth Factor-beta), Human Stem Cell Factor (SCF)/CD117 Ligand, Human Thrombopoietin (TPO, THPO), Human Transforming Growth Factor, Human TGF-alpha (Transforming Growth Factor-alpha, TGFa), Human TGF-beta 1 (Transforming Growth Factor-beta1, TGFb), Human TGF-beta 1.2 (Transforming Growth Factor-beta1, TGFb), Human TGF-beta 2 (Transforming Growth Factor-beta2, TGFb), Human TGF-beta 3 (Transforming Growth Factor-beta3, TGFb), Human VEGF (Vascular Endothelial Growth Factor), Human VEGF-121, Human VEGF-165, and Human VEGF-A. The above list would include sequence variants, analogues and agonists, including amino acid substitutions and/or extensions and/or deletions of the aforementioned factors (for example, IGF-I would include LR3-IGF-I, U.S. Patent No. 5,330,971) both naturally occurring and synthetic.

In some embodiments, the growth factor comprises insulin. Insulin can be recombinantly produced, isolated from natural sources, or synthetic. In some embodiments, the insulin is human recombinant insulin (EMD MILLIPORE CORPORATION). In some embodiments, insulin has a concentration of 0.5-15 mg/L upon hydration to form a cell culture medium. In some embodiments, the insulin has a concentration of about 3 mg/L upon hydration to form a cell culture medium.

In some embodiments, the one or more inorganic ions of the cell culture media supplement comprises trace metals selected from ammonium molybdate, chromium

potassium sulfate, cupric sulfate, lithium chloride, manganese sulfate, sodium metasilicate, ammonium paramolybdate, ammonium vanadium oxide, ferrous sulfate, nickel chloride, selenious acid, stannous chloride, zinc sulfate, and combinations thereof. In some embodiments, the ammonium molybdate is ammonium molybdate $4H_2O$; the chromium potassium sulfate is chromium potassium sulfate $12H_2O$, the cupric sulfate is cupric sulfate $5H_2O$, the lithium chloride is lithium chloride (anhydrous), the manganese sulfate is manganese sulfate H_2O and the sodium metasilicate is sodium metasilicate 9H2O. The chemicals are generally available from Sigma Aldrich and VWR.

In some embodiments, the one or more inorganic ions of the cell culture media supplement has the following final concentration upon hydration to form a cell culture medium:

i) 0.0005-0.01 mg/L of ammonium molybdate $4H_2O$;

ii) 0.0001-0.01 mg/L of chromium potassium sulfate $12H_2O$;

iii) 0.001-0.125 mg/L of cupric sulfate $5H_2O$;

iv) 0.001-0.1 mg/L of lithium chloride (anhydrous);

v) 0.00004-0.004 mg/L of manganese sulfate H_2O; and

vi) 0.04-4.2 mg/L of sodium metasilicate 9H2O.

In some embodiments, the one or more inorganic ions of the cell culture media supplement comprises a combination of ammonium molybdate $4H_2O$, chromium potassium sulfate $12H_2O$, cupric sulfate $5H_2O$, lithium chloride (anhydrous), manganese sulfate H_2O, and sodium metasilicate 9H2O. In some embodiments, the trace metals provide the following final concentration upon hydration to form a cell culture medium:

i) about 0.0037 mg/L of ammonium molybdate $4H_2O$;

ii) about 0.001 mg/L of chromium potassium sulfate $12H_2O$;

iii) about 0.0125 mg/L of cupric sulfate $5H_2O$;

iv) about 0.01 mg/L of lithium chloride (anhydrous);

v) about 0.000452 mg/L of manganese sulfate H_2O; and

vi) about 0.4263 mg/L of sodium metasilicate 9H2O.

The amino acid supplement of the cell culture media supplement can include any amino acid, including glycine, alanine, valine, leucine, isoleucine, arginine, lysine, aspartic acid, cysteine, cysteine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, asparagine, glutamine, histidine, and serine. In some embodiments, the amino acid supplement of the cell culture media supplement comprises a combination of asparagine, glutamine, histidine, and serine. Amino acids suitable for cell culture are generally available from Kyowa Hakko U.S.A., Research Organics, Inc. and Ajinomoto AminoScience LLC. In some embodiments, the amino acid supplement has the following final concentration upon hydration to form a cell culture medium:

i) 0.007-0.07 g/L asparagine H_2O;

ii) 0.25-2.5 g/L glutamine;

iii) 0.5-5.0 g/L histidine, free base; and

iv) 0.01-0.1 g/L serine.

In some embodiments, the amino acid supplement has the following final concentration upon hydration to form a cell culture medium:

i) about 0.0225 g/L asparagine H_2O;

ii) about 0.73 g/L glutamine;

iii) about 1.552 g/L histidine; and

iv) about 0.03676 g/L serine.

The buffer of the cell culture media supplement is not limiting. A "buffer" is a solution that resists changes in pH by the action of its acid-base conjugate components. Various buffers which can be employed depending, for example, on the desired pH of the buffer (and on the cells growth and metabolic properties, cell culture cultivation system, pH control and medium used) are described in Buffers. *A Guide for the Preparation and Use of Buffers in Biological Systems*, Gueffroy, D., ed. Calbiochem Corporation (1975). In one embodiment, the buffer has a pH in the

range from about 2 to about 9, alternatively from about 3 to about 8, alternatively from about 4 to about 7 alternatively from about 5 to about 7. Non-limiting examples of buffers that will control the pH in this range include MES, MOPS, MOPSO, Tris, HEPES, phosphate, acetate, citrate, succinate, and ammonium buffers, as well as combinations of these. In some embodiments, the buffer is selected from the group consisting of 3-(N-morpholino)propanesulfonic acid (MOPS) free acid, 3-(N-morpholino)propanesulfonic acid (MOPS) Na, hydroxyethyl piperazineethanesulfonic acid (HEPES) and sodium bicarbonate. In some embodiments, the formulation comprises a combination of MOPS free acid and MOPS Na. In some embodiments, the buffers of the cell culture media supplement provide the following final concentration when the compounded cell culture medium powder formulation is combined with water to form a cell culture medium:

i) 0.3-3 g/L MOPS free acid; and

ii) 1.0-10 g/L MOPS Na.

The anti-foaming agent of the invention is not limiting. In some embodiments, the anti-foaming agent of the compounded cell culture medium powder formulation comprises an ionic or non-ionic surfactant. Anti-foaming (also called 'defoaming') agents can inlcude oil, water, silicone, polyethylene glycol/ copolymers, and alkyl polyacrylates – based. Common examples include: Schill and Schelinger's Struktol SB2121 (a polyalkylene glycol), Schill and Schelinger's Struktol J673A (an alkoxylated fatty acid ester on a vegetable base), Fluka P2000 (a polypropylene glycol), Sigma Antifoam A (a 30% emulsion of silicone polymer) and Sigma Antifoam C (a 30% emulsion of silicone polymer) (Ref: Sarah J Routledge (2012); Beyond de-foaming: the effects of antifoams on bioprocess productivity. Computational and Structural Biotechnology Journal. 3(4) and references within). In some embodiments, the antifoaming agent is a polyol copolymer based on ethylene oxide and propylene oxide.

In some embodiments, the anti-foaming agent is Pluronic F68. Pluronic F-68 is a nonionic block copolymer with an average molecular weight of 8400, consisting of a center block of poly(oxypropylene) (20% by weight) and blocks of

poly(oxyethylene) at both ends. In some embodiments, other polyols can also be used and include nonionic block copolymers of poly(oxyethylene) and poly(oxypropylene) having molecular weights ranging from about 1000 to about 16,000.

In some embodiments, the anti-foaming agent is Pluronic F68 and has a concentration of 0.1-10 g/L when the compounded cell culture medium powder formulation is combined with water to form a cell culture medium. In some embodiments, the Pluronic F68 has a concentration of about 1 g/L when the compounded cell culture medium powder formulation is combined with water to form a cell culture medium.

In some embodiments, the compounded cell culture medium powder formulation comprises the following components which provide the following final concentration upon hydration to form a cell culture medium:

i) 0.0003-0.003 g/L biotin;

ii) 0.035-0.33 g/L calcium chloride;

iii) 0.003-0.03 g/L choline chloride;

iv) 0.0002-0.002 g/L cyanocobalamin (B12);

v) 1-10 g/L D+ mannose;

vi) 0.001-0.01 g/L D-calcium pantothenate;

vii) 0.3-3.0 g/L dextrose (anhydrous);

viii) 0.00003-0.0003 g/L DL-alpha-lipoic acid;

ix) 0.00002-0.00015 g/L ferric nitrate $9H_20$;

x) 0.0001-0.0015 g/L ferrous sulfate $7H_20$;

xi) 0.001-0.01 g/L folic acid;

xii) 0.007-0.20 g/L glycine;

xiii) 0.001-0.01 g/L hypoxanthine 2Na;

xiv) 0.005-0.05 g/L I-inositol;

xv) 0.003-0.03 g/L L-alanine;

xvi) 0.08-1.4 g/L L-arginine;

xvii) 0.006-0.16 g/L L-asparagine;

xviii) 0.005-0.10 g/L L-aspartic acid;

xix) 0.005-0.05 g/L L-cysteine HCl H_20;

xx) 0.02-0.2 g/L L-cystine 2HCl;

xxi) 0.005-0.15 g/L L-glutamic acid (anhydrous);

xxii) 0.02-0.6 g/L L-glutamine;

xxiii) 0.0003-0.003 g/L L-glutathione;

xxiv) 0.02-0.2 g/L L-histidine HCl;

xxv) 0.03-0.9 g/L L-isoleucine;

xxvi) 0.03-0.9 g/L L-leucine;

xxvii) 0.05-1.5 g/L L-lysine;

xxviii) 0.01-0.3 g/L L-methionine;

xxix) 0.02-0.6 g/L L-phenylaline;

xxx) 0.008-0.25 g/L L-proline;

xxxi) 0.009-0.25 g/L L-serine;

xxxii) 0.03-0.9 g/L L-threonine;

xxxiii) 0.006-0.16 g/L L-tryptophan;

xxxiv) 0.03-0.9 g/L L-tyrosine 2Na $2H_2O$;

xxxv) 0.03-0.9 g/L L-valine;

xxxvi) 0.01-0.18 g/L magnesium chloride

xxxvii) 0.02-0.12 g/L magnesium sulfate anhydrous;

xxxviii) 0.001-0.01 g/L niacinamide;

xxxix) 0.0005-0.005 g/L O-phoshphoryl-ethanolamine;

xl) 0.1-1.0 g/L potassium chloride;

xli) 0.00002-0.0002 g/L putrescine 2HCl;

xlii) 0.001-0.01 g/L pyridoxal HCl;

xliii) 0.00001-0.0001 g/L pyridoxine HCl,

xliv) 0.0001-0.001 g/L riboflavin,

xlv) 2.0-15 g/L sodium chloride,

xlvi) 0.02-0.2 g/L sodium phosphate monobasic H_20,

xlvii) 0.02-0.2 g/L sodium phosphate dibasic anhydrous,

xlviii) 0.015-0.15 g/L sodium pyruvate,

xlix) 0.001-0.01 g/L thiamine HCl,

l) 0.0001-0.001 g/L thymidine,

li) 0.00015-0.0015 g/L zinc sulfate $7H_20$,

lii) 0.0000006-0.000006 g/L cupric sulfate $5H_2O$,

liii) 0.0000005-0.000008 g/L selenium dioxide,

liv) 0.00001-0.0001 g/L linoleic acid,

lv) 0.0001-0.001 g/L beta-mercaptoethanol;

lvi) 0.0003-0.005 g/L ethanolamine FB;

lvii) 0.5-5 g/L MgCl2;

lviii) 0.5-15 mg/L insulin;

lix) 0.0005-0.01 mg/L of ammonium molybdate $4H_2O$;

lx) 0.0001-0.01 mg/L of chromium potassium sulfate $12H_2O$;

lxi) 0.001-0.125 mg/L of cupric sulfate $5H_2O$;

lxii) 0.001-0.1 mg/L of lithium chloride (anhydrous);

lxiii) 0.00004-0.004 mg/L of manganese sulfate H_2O;

lxiv) 0.04-4.2 mg/L of sodium metasilicate 9H2O;

lxv) 0.007-0.07 g/L asparagine H_2O;

lxvi) 0.25-2.5 g/L glutamine;

lxvii) 0.5-5.0 g/L histidine, free base;

lxviii) 0.01-0.1 g/L serine;

lxix) 0.3-3 g/L MOPS free acid;

lxx) 1.0-10 g/L MOPS Na; and

lxxi) 0.1-10 g/L Pluronic F68.

In another embodiment, the invention provides a method of making the compounded cell culture medium powder formulation of the invention, comprising combining the components of the basal medium powder; one or more salts; one or more growth factors; one or more inorganic ions; an amino acid supplement comprising one or more of asparagine, glutamine, histidine, and serine; one or more buffers; and one or more anti-foaming agents. The order of addition of the components of the compounded cell culture medium powder formulation is not limiting. In one embodiment, each component of the composition is added individually to make the compounded cell culture medium powder formulation. In some embodiments, the basal media is first made as a batch and then is combined with the other components that make up the culture media supplement. In some embodiments, the cell culture media supplement can be made as a batch, and later combined with a batched basal media powder or combined with the components of the basal media powder by a sequential addition to make the compounded cell culture medium powder formulation.

A cell culture medium can be prepared using the compounded cell culture medium powder formulation of the invention by performing a hydration step. In another aspect, the invention provides a method of making a cell culture medium for growing cells, comprising contacting the compounded cell culture medium powder formulation with water, thereby making a cell culture medium for growing cells. In one embodiment, the cells are mammalian cells. In some embodiments, the components are substantially dissolved in the water. In some embodiments, a tank is filled with water and the compounded cell culture medium powder formulation is

added to the tank. The user can optionally perform quality control process steps to make sure that the powder is dissolved, by mixing, and testing osmolality, conductivity, and the pH to ensure all of the components are present in the targeted amounts.

In one embodiment, the invention provides a method of making a cell culture medium for growing cells, comprising substantially dissolving the compounded cell culture medium powder formulation with water, and optionally further comprising combining a solution comprising $FeSO_4$ $7H_2O$ and a chelating agent. In some embodiments, the water and solution comprising $FeSO_4$ $7H_2O$ and a chelating agent are mixed first, followed by addition of the compounded cell culture medium powder formulation. In some embodiments, the chelating agent is ethylenediaminetetraacetic acid (EDTA). In some embodiments, the $FeSO_4$ $7H_2O$ and EDTA have the following final concentration in the cell culture medium: 0.004-0.04 g/L $FeSO_4$ $7H_2O$; and 0.006-0.06 g/L EDTA. In some embodiments, the $FeSO_4$ $7H_2O$ and EDTA have the following final concentration in the cell culture medium: about 0.0138g/L $FeSO_4$ $7H_2O$; and about 0.018625g/L EDTA.

In another embodiment, the invention provides a method of culturing cells, comprising, comprising contacting cells with a cell culture medium of the invention and culturing the cells in the medium for a period of time. In some embodiments, the cells are incubated in fed-batch or semi-batch culture. In some embodiments, the cells are incubated in a perfusion culture.

In some embodiments, the cell culture medium is for expressing a protein of interest. In another embodiment, the invention provides a method of producing a protein of interest, comprising contacting cells expressing the protein of interest with a cell culture medium; culturing the cells in the medium for a period of time; and isolating the protein of interest from the cell culture medium.

In some embodiments the protein of interest is selected from the group consisting of coagulation Factor VIII (FVIII), and functional variants and fragments thereof. In some embodiments, the FVIII polypeptides include allelic variations, glycosylated versions, modifications and fragments resulting in derivatives of FVIII

so long as they contain the functional segment of human FVIII and the essential, characteristic human FVIII functional activity.

In some embodiments, the FVIII molecules useful for expression using the compounded media of the present invention include the full length protein, precursors of the protein, subunits or fragments of the protein, and variants and antigenic fragments thereof. Reference to FVIII is meant to include all potential forms of such proteins.

Examples of recombinant FVIII include Recombinate™ and Advate®, both manufactured and sold by Baxter Healthcare Corporation; ReFacto®, a B-domain deleted form of FVIII manufactured and sold by Wyeth Corporation; and KOGENATE, manufactured and sold by Bayer Corporation. In some embodiments, the FVIII polypeptides to be expressed comprise full-length human FVIII. In some embodiments, the full length FVIII comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and a combination thereof, although allelic variants are possible. As a secreted protein, FVIII contains a signal sequence that is proteolytically cleaved during the translation process. Following removal of the 19 amino acid signal sequence, the first amino acid of the secreted FVIII product is an alanine.

In some embodiments, the human FVIII is B-domain deleted FVIII (BDD). As used herein, BDD is characterized by having the amino acid sequence which contains a deletion of all but 14 amino acids of the B-domain of FVIII. The first 4 amino acids of the B-domain (SEQ ID NO:3) are linked to the 10 last residues of the B-domain (NPPVLKRHQR, SEQ ID NO:4). In some embodiments, the BDD FVIII comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 5 and SEQ ID NO: 6 and a combination thereof.

In some embodiments, FVIII can be modified with a biocompatible polymer, such as PEG. Pegylated forms of Factor VIII are disclosed in WO 2006/053299 and U.S. Patent Application Pub. No. 20060115876, which are incorporated by reference herein.

In the examples of FVIII that follow, the FVIII muteins are named in a manner conventional in the art. As used herein, a "mutein" is a genetically engineered protein arising as a result of a laboratory induced mutation to a protein or

polypeptide The convention for naming mutants is based on the amino acid sequence for the mature, full length Factor VIII as provided in SEQ ID NO:2.

As is conventional and used herein, when referring to mutated amino acids in BDD FVIII, the mutated amino acid is designated by its position in the sequence of full-length FVIII. For example, a particular mutein is designated K1808C because it changes the lysine (K) at the position analogous to 1808 in the full-length sequence to cysteine (C). In some embodiments, for the mutants discussed below, a cysteine replaces the natural amino acid at the designated location of the full length FVIII or the B-domain deleted FVIII, and a biocompatible polymer, such as PEG, is attached to the cysteine residue.

The predefined site for covalent binding of a biocompatible polymer, such as PEG, is best selected from sites exposed on the surface of the polypeptide that are not involved in FVIII activity or involved in other mechanisms that stabilize FVIII in vivo, such as binding to vWF. Such sites are also best selected from those sites known to be involved in mechanisms by which FVIII is deactivated or cleared from circulation. Selection of these sites is discussed in detail below. Preferred sites include an amino acid residue in or near a binding site for (a) low density lipoprotein receptor related protein, (b) a heparin sulphate proteoglycan, (c) low density lipoprotein receptor and/or (d) factor VIII inhibitory antibodies. By "in or near a binding site" means a residue that is sufficiently close to a binding site such that covalent attachment of a biocompatible polymer to the site would result in steric hindrance of the binding site. Such a site is expected to be within 20 Angstroms of a binding site, for example.

In one embodiment, the biocompatible polymer is covalently attached to the functional factor VIII polypeptide at an amino acid residue in or near (a) a factor VIII clearance receptor, (b) a binding site for a protease capable of degradation of factor VIII and/or (c) a binding site for factor VIII inhibitory antibodies. The protease may be activated protein C (APC). In another embodiment, the biocompatible polymer is covalently attached at the predefined site on the functional factor VIII polypeptide such that binding of low-density lipoprotein receptor related protein to the polypeptide is less than to the polypeptide when it is not conjugated, and preferably more than twofold less. In one embodiment, the biocompatible

polymer is covalently attached at the predefined site on the functional factor VIII polypeptide such that binding of heparin sulphate proteoglycans to the polypeptide is less than to the polypeptide when it is not conjugated, and preferably is more than twofold less. In a further embodiment, the biocompatible polymer is covalently attached at the predefined site on the functional factor VIII polypeptide such that binding of factor VIII inhibitory antibodies to the polypeptide is less than to the polypeptide when it is not conjugated, preferably more than twofold less than the binding to the polypeptide when it is not conjugated. In another embodiment, the biocompatible polymer is covalently attached at the predefined site on the functional factor VIII polypeptide such that binding of low density lipoprotein receptor to the polypeptide is less than to the polypeptide when it is not conjugated, preferably more than twofold less. In another embodiment, the biocompatible polymer is covalently attached at the predefined site on the functional factor VIII polypeptide such that a plasma protease degrades the polypeptide less than when the polypeptide is not conjugated. In a further embodiment, the degradation of the polypeptide by the plasma protease is more than twofold less than the degradation of the polypeptide when it is not conjugated as measured under the same conditions over the same time period.

LRP, LDL receptor, or HSPG binding affinity for FVIII can be determined using surface plasmon resonance technology (Biacore). For example, FVIII can be coated directly or indirectly through a FVIII antibody to a Biacore chip, and varying concentrations of LRP can be passed over the chip to measure both on-rate and off-rate of the interaction (Bovenschen N. *et al.*, 2003, *J. Biol. Chem.* 278(11), pp. 9370-7). The ratio of the two rates gives a measure of affinity. A two-fold, preferably five-fold, more preferably ten-fold, and even more preferably 30-fold decrease in affinity upon PEGylation would be desired.

Degradation of a FVIII by the protease APC can be measured by any of the methods known to those of skill in the art.

In one embodiment, the biocompatible polymer is covalently attached to the polypeptide at one or more of the FVIII (SEQ ID NO:2) amino acid positions 81, 129, 377, 378, 468, 487, 491, 504, 556, 570, 711, 1648, 1795, 1796, 1803, 1804, 1808, 1810, 1864, 1903, 1911, 2091, 2118 and 2284. In another embodiment, the

biocompatible polymer is covalently attached to the polypeptide at one or more of factor VIII (SEQ ID NO:2) amino acid positions 377, 378, 468, 491, 504, 556, 1795, 1796, 1803, 1804, 1808, 1810, 1864, 1903, 1911 and 2284 and (1) the binding of the conjugate to low-density lipoprotein receptor related protein is less than the binding of the unconjugated polypeptide to the low-density lipoprotein receptor related protein; (2) the binding of the conjugate to low-density lipoprotein receptor is less than the binding of the unconjugated polypeptide to the low-density lipoprotein receptor; or (3) the binding of the conjugate to both low-density lipoprotein receptor related protein and low-density lipoprotein receptor is less than the binding of the unconjugated polypeptide to the low-density lipoprotein receptor related protein and the low-density lipoprotein receptor. In one embodiment, residue 1804 in a B-domain deleted FVIII is mutated to cysteine and conjugated to PEG.

In a further embodiment, the biocompatible polymer is covalently attached to the polypeptide at one or more of FVIII (SEQ ID NO:2) amino acid positions 377, 378, 468, 491, 504, 556 and 711 and the binding of the conjugate to heparin sulphate proteoglycan is less than the binding of the unconjugated polypeptide to heparin sulphate proteoglycan. In a further embodiment, the biocompatible polymer is covalently attached to the polypeptide at one or more of the factor VIII (SEQ ID NO:2) amino acid positions 81, 129, 377, 378, 468, 487, 491, 504, 556, 570, 711, 1648, 1795, 1796, 1803, 1804, 1808, 1810, 1864, 1903, 1911, 2091, 2118 and 2284 and the conjugate has less binding to factor VIII inhibitory antibodies than the unconjugated polypeptide. In a further embodiment, the biocompatible polymer is covalently attached to the polypeptide at one or more of the factor VIII (SEQ ID NO:2) amino acid positions 81, 129, 377, 378, 468, 487, 491, 504, 556, 570, 711, 1648, 1795, 1796, 1803, 1804, 1808, 1810, 1864, 1903, 1911, 2091, 2118 and 2284, and preferably at one or more of positions 377, 378, 468, 491, 504, 556, and 711 and the conjugate has less degradation from a plasma protease capable of factor VIII degradation than does the unconjugated polypeptide. More preferred, the plasma protease is activated protein C.

In a further embodiment, the biocompatible polymer is covalently attached to B-domain deleted factor VIII at amino acid position 129, 491, 1804, and/or 1808, more preferably at 491 or 1808. In a further embodiment, the biocompatible polymer

is attached to the polypeptide at factor VIII amino acid position 1804 and comprises polyethylene glycol. Preferably, the one or more predefined sites for biocompatible polymer attachment are controlled by site specific cysteine mutation.

One or more sites, preferably one or two, on the functional factor VIII polypeptide may be the predefined sites for polymer attachment. In particular embodiments, the polypeptide is mono-PEGylated or diPEGylated.

The invention also relates to a method for the preparation of the conjugate comprising mutating a nucleotide sequence that encodes for the functional factor VIII polypeptide to substitute a coding sequence for a cysteine residue at a pre-defined site; expressing the mutated nucleotide sequence to produce a cysteine enhanced mutein; purifying the mutein; reacting the mutein with the biocompatible polymer that has been activated to react with polypeptides at substantially only reduced cysteine residues such that the conjugate is formed; and purifying the conjugate. In another embodiment, the invention provides a method for site-directed PEGylation of a factor VIII mutein comprising: (a) expressing a site-directed factor VIII mutein wherein the mutein has a cysteine replacement for an amino acid residue on the exposed surface of the factor VIII mutein and that cysteine is capped; (b) contacting the cysteine mutein with a reductant under conditions to mildly reduce the cysteine mutein and to release the cap; (c) removing the cap and the reductant from the cysteine mutein; and (d) at least about 5 minutes, and preferably at least 15 minutes, still more preferably at least 30 minutes after the removal of the reductant, treating the cysteine mutein with PEG comprising a sulfhydryl coupling moiety under conditions such that PEGylated factor VIII mutein is produced. The sulfhydryl coupling moiety of the PEG is selected from the group consisting of thiol, triflate, tresylate, aziridine, oxirane, S-pyridyl and maleimide moieties, preferably maleimide.

In one embodiment, one or more surface BDD amino acids is replaced with a cysteine, producing the cysteine mutein in a mammalian expression system, reducing a cysteine which has been capped during expression by cysteine from growth media, removing the reductant to allow BDD disulfides to reform, and reacting with a cysteine-specific biocompatible polymer reagent, such as such as PEG-maleimide. Examples of such reagents are PEG-maleimide with PEG sizes

such as 5, 22, or 43 kD available from Nektar Therapeutics of San Carlos, Calif. under Nektar catalog numbers 2D2M0H01 mPEG-MAL MW 5,000 Da, 2D2M0P01 mPEG-MAL MW 20 kD, 2D3X0P01 mPEG2-MAL MW 40 kD, respectively, or 12 or 33 kD available from NOF Corporation, Tokyo, Japan under NOF catalog number Sunbright ME-120MA and Sunbright ME-300MA, respectively. The PEGylated product is purified using ion-exchange chromatography to remove unreacted PEG and using size-exclusion chromatography to remove unreacted BDD. This method can be used to identify and selectively shield any unfavorable interactions with FVIII such as receptor-mediated clearance, inhibitory antibody binding, and degradation by proteolytic enzymes. We noted that the PEG reagent supplied by Nektar or NOF as 5 kD tested as 6 kD in our laboratory, and similarly the PEG reagent supplied as linear 20 kD tested as 22 kD, that supplied as 40 kD tested as 43 kD and that supplied as 60 kD tested as 64 kD in our laboratory. To avoid confusion, we use the molecular weight as tested in our laboratory in the discussion herein, except for the 5 kD PEG, which we report as 5 kD as the manufacturer identified it.

In addition to cysteine mutations at positions 491 and 1808 of BDD (disclosed above), positions 487, 496, 504, 468, 1810, 1812, 1813, 1815, 1795, 1796, 1803, and 1804 were mutated to cysteine to potentially allow blockage of LRP binding upon PEGylation. Also, positions 377, 378, and 556 were mutated to cysteine to allow blockage of both LRP and HSPG binding upon PEGylation. Positions 81, 129, 422, 523, 570, 1864, 1911, 2091, and 2284 were selected to be equally spaced on BDD so that site-directed PEGylation with large PEGs (>40 kD) at these positions together with PEGylation at the native glycosylation sites (41, 239, and 2118) and LRP binding sites should completely cover the surface of BDD and identify novel clearance mechanism for BDD.

In one embodiment, the cell culture medium contains cysteines that "cap" the cysteine residues on the mutein by forming disulfide bonds. In the preparation of the conjugate, the cysteine mutein produced in the recombinant system is capped with a cysteine from the medium and this cap is removed by mild reduction that releases the cap before adding the cysteine-specific polymer reagent. Other methods known

in the art for site-specific mutation of FVIII may also be used, as would be apparent to one of skill in the art.

In some embodiments, the FVIII is selected from wild-type FVIII, B-domain deleted FVIII and FVIII conjugated with a biocompatible polymer. In some embodiments, the biocompatible polymer is polyethylene glycol (PEG). In some embodiments, the PEG is covalently attached to the polypeptide at one or more of the factor VIII amino acid positions 81, 129, 377, 378, 468, 487, 491, 504, 556, 570, 711, 1648, 1795, 1796, 1803, 1804, 1808, 1810, 1864, 1903, 1911, 2091, 2118 and 2284.

While the invention has been described with reference to certain particular examples and embodiments herein, those skilled in the art will appreciate that various examples and embodiments can be combined for the purpose of complying with all relevant patent laws (e.g., methods described in specific examples can be used to describe particular aspects of the invention and its operation even though such are not explicitly set forth in reference thereto).

EXAMPLE 1

The current method of media preparation for producing Factor VIII involves the addition of several add-back solutions and dry powder components/mixes in order to construct their cell culture medium. Several related powder variations were evaluated that included some or all of the add-backs (milled into the DPM) in order to identify a more complex (i.e., fewer add-backs) powder formulation that has acceptable growth and performance characteristics. The goal is to streamline medium formulation process and reduce the supply chain complexity (as seen in Table 1).

For producing a powdered media formulation suitable for producing a pegylated Factor VIII, an optimized FVIII powder formulation was blended with an amino acid powder blend (3x AA mix) into one formulation and the characteristics were evaluated.

Materials/Methods

Materials

The balance was a Sartorius MSA124S; the osmometer was Advanced Instruments, Model 3300; the pH readings were taken with a ThermoOrion Model 720A; turbidity readings measured on a Hach 2100N Turbidimeter; Insulin UPLC analysis was done via Acquity H-Class, Acquity BEH300 C4 column; Insulin ELISA quantitation was done using a Millipore ELISA kit; Amino acid quantitation was done via Waters HPLC, Zorbax Eclipse AAA column; the ICP analyses were done on an Agilent 720-ES ICP-OES.

Methods

The DPM versions and all associated add-back solutions and powders were manufactured by SAFC's Immediate Advantage department. The add-back powders and solutions are summarized in Table 1. The versions, description of the powders, and SAFC product numbers are summarized in Table 2.

Table 1: Current Media Formulation Materials

	Description	**Conc. In 1L**
	R3* w/o Phenol Red	**13.036g/L**
	rH-Insulin	**3.0mg/L (0.6mL/L)**
	MgCl2 Anhydrous*	**1.428g/L**
	10mM FeSO4/EDTA Soln. (TE #1)	**5mL/L**
	FeSO4 7H2O	0.0138g/L
	EDTA 2Na 2 H2O	0.018625g/L
	Trace Metal Solution #2	**0.1mL/L**
	Ammonium Molybdate 4H2O	0.0037mg/L
	Chromium Potassium Sulfate 12H2O	0.001mg/L

	Cupric Sulfate 5H2O	0.0125mg/L
	Lithium Chloride Anhydrous	0.01mg/L
	Manganese Sulfate H2O	0.000452mg/L
	Sodium Metasilicate 9H2O	0.4263mg/L
	Factor VIII (non-pegylated) Supplement	**7.856g/L**
	Asparagine H2O	0.0225g/L
	Glutamine	0.73g/L
	Histidine	1.552g/L
	Serine	0.03676g/L
	Lutrol (Pluronic F68)	1.0g/L
	MOPS Free Acid	1.0465g/L
	MOPS Na	3.468g/L
	3x AA Mix (for pegylated FVIII only)	**2.594g/L**

*See the formulation of Example 2, below.

Table 2: Various Factor VIII (non-pegylated) formulations evaluated

Version	Description
1	Base Media (control) – R3 without Phenol Red (68213C)
2	Base Media + All Additions
2.1	Base Media + All Additions; adjusted histidine HCl and FB
3	Base Media + All Additions except TE Solution #1
3.1	Base Media + All Additions except TE Solution #1; adjusted histidine HCl
3.2	Base Media + All Additions except TE Solution #1; adjusted histidine FB
3.3	Base Media + All Additions except TE Solution #1; adjusted histidine HCl and FB

4	Base Media + All Additions except amino acids mix
5	Base Media + All Additions except TE Solution #1 and amino acid mix
6	Base Media + All Additions except Lutrol and MOPS

The pegylated FVIII formulation was a combination of 22.29 g/L non-pegylated FVIII Version 3.3 mixed with 2.594 g/L 3x AA Production Mix.

Results

These versions were tested for the following characteristics:

1) pH and osmolality of the hydrated powder
2) Analysis of amino acid concentration
3) Analysis of Fe concentration
4) Analysis of insulin concentration

Tables 3A and **3B** summarize the data for the non-pegylated FVIII versions tested.

Table 3A: Finished product results for non-pegylated FVIII Versions 1-6

	Hydration		Amino Acid Data (Percent of control (Ver. 1); %)			
Version	**pH**	**Osmolality**	**Ser**	**Asn**	**Gln**	**His**
1	7.49	346	NA	NA	NA	NA
2	6.93	333	97.7	98.0	96.8	72.2
2.1	7.44	339	102.8	97.2	102.7	101.8
3	6.96	334	101.2	97.8	96.8	71.6
3.1	7.12	NA	NA	NA	NA	111.4
3.2	8.03	NA	NA	NA	NA	113.4
3.3	7.56	329	103.5	98.6	100.8	100.2
4	6.15	282	47.4	46.6	48.4	3.5
5	6.24	285	46.7	48.1	48.0	3.4
6	5.09	305	97.8	97.4	96.8	72.3

Table 3B: Finished product results for non-pegylated FVIII Versions 1-6

	ICP Data	Insulin	
Version	Fe (ppm)	HPLC (mg/L)	ELISA (retests if applicable) (mg/L)
Theoretical	4.0	3.00	3.00
1	4.1	3.60	1.26, 2.62
2	2.9	3.05	
2.1	2.8	3.40	1.4
3	0.4	3.04	
3.1	NA		
3.2	NA		
3.3	0.33	3.95	0.880, 1.26, 3.70
4	2.9		
5	0.4		
6	3.0		

The use of HPLC for insulin quantitation is more accurate and precise than using the Millipore ELISA kit for insulin. The HPLC method is extremely similar to the USP HPLC method for insulin quantitation, and thus is robust and rugged, and its accuracy and precision have been established. In contrast, the ELISA method is designed for simple insulin formulations, and is not likely optimized for use in such complex formulations found in cell culture. It is likely the inconsistent results seen with the ELISA test is due to component interference with insulin binding (or competitive inhibition), therefore making the ELISA test sub-optimal for accurate quantitation of insulin in such complex cell culture media.

Table 4 outlines the iron residue left on the 0.22µm filter paper used to filter 1L of Version 1, Version 2.1, and Version 3.3. The result of this iron testing was the basis for selecting the Version 3.3 as the formulation for use.

Table 4: Iron amounts from rinsed 0.22µm filter papers used to individually filter various versions of non-pegylated FVIII

	Control						
	Blank	Version 2.1		Version 3.3		Version 1	
Trace Element	Filter Paper (µg)	Prep A (µg)	Prep B (µg)	Prep A (µg)	Prep B (µg)	Prep A (µg)	Prep B (µg)
Fe	<10	190	170	1	1	2	1

Table 5 summarizes the data for the KG-N version tested.

Table 5: Finished product results for pegylated FVIII version

	Hydration		Amino Acid Data (Percent of control; %)			
Lot #	pH	Osmolality	Ser	Asn	Gln	His
13D530	7.57	354	99.7	97.4	96.4	98.9

	ICP Data	Insulin	
Lot #	Fe (ppm)	HPLC (mg/L)	ELISA (retests if applicable) (mg/L)
13D530	0.33	3.62	0.890, 1.09

Conclusions

The final version of the non-pegylated FVIII was Version 3.3. This is the complete media minus the majority (97.1%) of iron and all of the EDTA in the formulation. This iron/EDTA-free dry powder formulation (utilizing an iron/EDTA liquid add-back) was selected as the formulation due to the discovery that Version 2.1 (containing iron and EDTA milled into the DPM) did not properly chelate (i.e., solubilize) the iron upon hydration, and thus the iron was being filtered out of the solution (**Table 4**).

The analytical evaluation of Version 3.3 measured insulin concentration, amino acid concentrations, and pH (upon hydration) that was not significantly different than a fully formulated Version 1 (the control).

The pegylated FVIII formulation is the non-pegylated FVIII formulation fortified with additional amino acids.

EXAMPLE 2

This example shows an exemplary basal medium which can be enhanced by further compounding into the compounded media formulation of the invention. In this example, an indicator such as phenol red, is included, however, the indicator can be omitted.

Component	Units/Liter
L-Alanine, USP/EP	0.01336 g
L-Arginine, HCl, USP	0.27435 g
L-Asparagine H20	0.0225 g
L-Aspartic acid USP	0.01995 g
Biotin, USP	0.001 g
Calcium chloride, anhydrous	0.11665 g
D-Calcium Pantothenate, USP	0.00312 g
Choline Chloride, USP	0.00998 g
Cupric Sulfate, $5H_20$, ACS	0.000001275 g
cyanocobalamin, lisp	0.00068 g
L-Cysteine, HCl , H_2O, USP, EP	0.01756 g
L-Cysteine, 2HC1	0.06256 g
Dextrose, Anhydrous, ACS	1.0 g
Ethanolamine, FB	0.0012 g

Ortho Phosphorylethanolamine	0.0014 g
Ferric Nitrate, $9H_20$, ACS	0.00005 g
Ferrous Sulfate, $7H_20$, ACS	0.000417 g
Folic Acid, USP	0.00366 g
L-Glutamic Acid, EP	0.02206 g
L-Glutamine, USP	0.73 g
L-Glutathione, Reduced	0.001 g
Glycine USP, EP, JP	0.0262 g
L-Histidine, HCl, H_20, EP	0.07348 g
Hypoxanthine 2Na	0.0027 g
i-Inositol	0.01451 g
L-Isoleucine, USP,EP, JP	0.1057 g
L-Leucine, USP, EP, JP	0.11096 g
Linoleic acid	0.000042 g
DL-Alpha-Lipoic Acid	0.000103 g
L-Lysine, HCl, USP, EP, JP	0.16385 g
Magnesium Chloride, $6H_20$ ACS	0.061 g
Magnesium Sulfate, Anhydrous, USP	0.04884 g
2-Mercaptoethanol	0.00039065 g
L-Methionine USP, EP, JP	0.03224 g
Phenol Red, Na salt, ACS	0.0069 g
Niacinamide, USP	0.00302 g

L-Phenylalanine, USP, EP	0.06748 g
Potassium Chloride, USP	0.31182 g
L-Proline, USP, EP	0.02875 g
Putrescine, 2HC1	0.000081 g
Pyridoxal HCl	0.003 g
Pyridoxine, HCl, USP	0.000031 g
Riboflavin, USP	0.000319 g
Selenium Dioxide	0.00000222 g
L-Serine, USP, EP	0.03676 g
Sodium Chloride, ACS	6.1234 g
Sodium Phosphate, Dibasic, Anhydrous, USP	0.07099 g
Sodium Phosphate, Monobasic, H_20, USP	0.0625 g
Sodium Pyruvate	0.055 g
Thiamine, HCl, USP	0.00317 g
L-Threonine, USP, EP, JP	0.10156 g
Thymidine	0.000364 g
L-Tryptophan, USP, EP, JP	0.01902 g
L-Tyrosine, 2Na, $2H_20$	0.10771 g
L-Valine, USP, EP, JP	0.09866 g
Zinc Sulfate, 7 H_20, ACS	0.000432 g
D-Mannose	3.0 g
Total	13.042543120 g/L

EXAMPLE 3

This example describes an exemplary embodiment of a compounded media powder formulation.

Component	Units/Liter
L-Alanine, USP/EP	0.01336 g
Ammonium Molybdate, $4H_20$	0.0000037 g
L-Arginine, HCl, USP	0.27435 g
L-Asparagine H_20	0.045 g
L-Aspartic acid USP	0.01995 g
Biotin, USP	0.001 g
Calcium chloride, anhydrous	0.11665 g
D-Calcium Pantothenate, USP	0.00312 g
Choline Chloride, USP	0.00998 g
Chromic Potassium Sulfate, $12H_20$	0.000001 g
Cupric Sulfate, $5H_20$, ACS	0.00001375 g
cyanocobalamin, USP	0.00068 g
L-Cysteine, HCl , H_2O, USP, EP	0.01756 g
L-Cysteine, 2HC1	0.06256 g
Dextrose, Anhydrous, ACS	1.0 g
Ethanolamine, FB	0.0012 g

Ortho Phosphorylethanolamine	0.0014 g
Ferric Nitrate, $9H_20$, ACS	0.00005 g
Ferrous Sulfate, $7H_20$, ACS	0.000417 g
Folic Acid, USP	0.00366 g
L-Glutamic Acid, EP	0.02206 g
L-Glutamine, USP	1.46 g
L-Glutathione, Reduced	0.001 g
Glycine . USP, EP, JP	0.0262 g
L-Histidine, FB, USP, EP	1.552 g
L-Histidine, HCl, H20, EP	0.07348 g
Hypoxanthine 2Na	0.0027 g
i-Inositol	0.01451 g
L-Isoleucine, USP,EP, JP	0.1057 g
L-Leucine, USP, EP, JP	0.11096 g
Linoleic acid	0.000042 g
DL-Alpha-Lipoic Acid	0.000103 g
Lithium Chloride	0.00001 g
L-Lysine, HCl, USP, EP, JP	0.16385 g
Magnesium Chloride, Anhydrous	1.456951840 g
Magnesium Sulfate, Anhydrous, USP	0.04884 g
2-Mercaptoethanol	0.000390650 g
L-Methionine USP, EP, JP	0.03224 g

Niacinamide, USP	0.00302 g
L-Phenylalanine, USP, EP	0.06748 g
Lutrol (R) P-68, NF	1.0 g
Potassium Chloride, USP	0.31182 g
L-Proline, USP, EP	0.02875 g
Putrescine, 2HC1	0.000081 g
Pyridoxal. HCl	0.003 g
Pyridoxine, HCl, USP	0.000031 g
Riboflavin, USP	0.000319 g
Selenium Dioxide	0.00000222 g
L-Serine, USP, EP	0.07352 g
Sodium Chloride, ACS	6.1234 g
Sodium Meta-Silicate, $9H_20$	0.0004263 g
Sodium Phosphate, Dibasic, Anhydrous	0.07099 g
Sodium Phosphate, Monobasic, H_20, USP	0.0625 g
Sodium Pyruvate	0.055 g
Thiamine, HCl, USP	0.00317 g
L-Threonine, USP, EP, JP	0.10156 g
Thymidine	0.000364 g
L-Tryptophan, USP, EP, JP	0.01902 g
L-Tyrosine, 2Na, $2H_20$	0.10771 g
L-Valine, USP, EP, JP	0.09866 g
Zinc Sulfate, 7 H_20, ACS	0.000432 g

D-Mannose	3.0 g
MOPS, Free Acid, BPC (Sigma M3183)	1.0465 g
MOPS, Sodium, BPC (Sigma M9024)	3.4680 g
Insulin, human recombinant, ACF, USP (#4506)	0.003 g
Ammonium Molybdate $4H_2O$	0.0037 mg
Chromium Potassium Sulfate $12H_2O$	0.001 mg
Cupric Sulfate $5H_2O$	0.0125 mg
Lithium Chloride Anhydrous	0.01 mg
Manganese Sulfate H_2O	0.000452mg
Sodium Metasilicate $9H_2O$	0.4263 mg
Total	22.290808460 g/L

The compounded cell culture medium powder formulation was hydrated in 1 L of double-deionized sterile H_20 in a hydration tank. The powder was added to the water followed by mixing to ensure adequate dispersion and dissolution. Next, a solution comprising FeSO4 7H2O and ethylenediaminetetraacetic acid (EDTA) was added to the tank. The FeSO4 7H2O and EDTA have the following final concentration in the cell culture medium: about 0.0138g/L FeSO4 7H2O; and about 0.018625g/L EDTA. The cell culture media was then stored at 4 °C for later use.

EXAMPLE 4

This example describes an exemplary compounded media powder formulation.

Component	Units/Liter
L-Alanine, USP/EP	0.01336 g
Ammonium Molybdate, $4H_20$	0.0000037 g

L-Arginine, HCl, USP	0.82305 g
L-Asparagine H_20	0.135 g
L-Aspartic acid USP	0.05985 g
Biotin, USP	0.001 g
Calcium chloride, anhydrous	0.11665 g
D-Calcium Pantothenate, USP	0.00312 g
Choline Chloride, USP	0.00998 g
Chromic Potassium Sulfate, $12H_20$	0.000001 g
Cupric Sulfate, $5H_20$, ACS	0.00001375 g
cyanocobalamin, USP	0.00068 g
L-Cysteine, HCl, H_2O, USP, EP	0.01756 g
L-Cysteine, 2HCl	0.06256 g
Dextrose, Anhydrous, ACS	1.0 g
Ethanolamine, FB	0.0012 g
Ortho Phosphorylethanolamine	0.0014 g
Ferric Nitrate, $9H_20$, ACS	0.00005 g
Ferrous Sulfate, $7H_20$, ACS	0.000417 g
Folic Acid, USP	0.00366 g
L-Glutamic Acid, EP	0.06618 g
L-Glutamine, USP	1.46 g

L-Glutathione, Reduced	0.001 g
Glycine . USP, EP, JP	0.07878 g
L-Histidine, FB, USP, EP	1.552 g
L-Histidine, HCl, H20, EP	0.07348 g
Hypoxanthine 2Na	0.0027 g
i-Inositol	0.01451 g
L-Isoleucine, USP,EP, JP	0.3171 g
L-Leucine, USP, EP, JP	0.33288 g
Linoleic acid	0.000042 g
DL-Alpha-Lipoic Acid	0.000103 g
Lithium Chloride	0.00001 g
L-Lysine, HCl, USP, EP, JP	0.49155 g
Magnesium Chloride, Anhydrous	1.456951840 g
Magnesium Sulfate, Anhydrous, USP	0.04884 g
2-Mercaptoethanol	0.000390650 g
L-Methionine USP, EP, JP	0.09672 g
Niacinamide, USP	0.00302 g
L-Phenylalanine, USP, EP	0.20244 g
Lutrol (R) P-68, NF	1.0 g
Potassium Chloride, USP	0.31182 g
L-Proline, USP, EP	0.08625 g
Putrescine, 2HC1	0.000081 g

Pyridoxal. HCl	0.003 g
Pyridoxine, HCl, USP	0.000031 g
Riboflavin, USP	0.000319 g
Selenium Dioxide	0.00000222 g
L-Serine, USP, EP	0.22052 g
Sodium Chloride, ACS	6.1234 g
Sodium Meta-Silicate, $9H_20$	0.0004263 g
Sodium Phosphate, Dibasic, Anhydrous	0.07099 g
Sodium Phosphate, Monobasic, H_20, USP	0.0625 g
Sodium Pyruvate	0.055 g
Thiamine, HCl, USP	0.00317 g
L-Threonine, USP, EP, JP	0.30468 g
Thymidine	0.000364 g
L-Tryptophan, USP, EP, JP	0.05706 g
L-Tyrosine, 2Na, 2H20	0.32313 g
L-Valine, USP, EP, JP	0.29598 g
Zinc Sulfate, 7 H20, ACS	0.000432 g
D-Mannose	3.0 g
MOPS, Free Acid, BPC (Sigma M3183)	1.0465 g
MOPS, Sodium, BPC (Sigma M9024)	3.4680 g
Insulin, human recombinant, ACF, USP (#4506)	0.003 g
Ammonium Molybdate $4H_2O$	0.0037 mg
Chromium Potassium Sulfate $12H_2O$	0.001 mg

Cupric Sulfate $5H_2O$	0.0125 mg
Lithium Chloride Anhydrous	0.01 mg
Manganese Sulfate H_2O	0.000452mg
Sodium Metasilicate $9H_2O$	0.4263 mg
Total	24.884908460 g/L

The compounded cell culture medium powder formulation was hydrated in 1 L of double-deionized sterile H_20 in a hydration tank. The powder was added to the water followed by mixing to ensure adequate dissolution. Next, a solution comprising $FeSO_4$ $7H_2O$ and ethylenediaminetetraacetic acid (EDTA) was added to the tank. The $FeSO_4$ $7H_2O$ and EDTA have the following final concentration in the cell culture medium: about 0.0138g/L $FeSO_4$ $7H_2O$; and about 0.018625g/L EDTA. The cell culture media was then stored at 4 °C for later use.

Feasibility of using the (further) compounded medium is demonstrated along two levels: (i) concentration determination following hydration and (ii) cell culture performance studies. Hydration studies show that the concentrations of key media components (in the further compounded medium) are in agreement with the theoretical (expected) values as well as with those in liquid medium prepared the standard way (See Example 1). 1L perfusion cell culture studies further demonstrate comparable cell culture performance (growth and metabolism) and recombinant protein production (potency/titer) when using either medium preparation method (Fig. 3).

While there have been shown and described what are presently believed to be the preferred embodiments of the present invention, those skilled in the art will realize that other and further embodiments can be made without departing from the spirit and scope of the invention described in this application, and this application includes all such modifications that are within the intended scope of the claims set forth herein. All patents and publications mentioned and/or cited herein are incorporated by reference to the same extent as if each individual publication was

specifically and individually indicated as having been incorporated by reference in its entirety.

WHAT IS CLAIMED IS:

1. A compounded cell culture medium powder formulation comprising a basal medium powder and a cell culture media supplement, wherein the cell culture media supplement comprises

    i) one or more salts;

    ii) one or more growth factors;

    iii) one or more inorganic ions;

    iv) an amino acid supplement comprising one or more of asparagine, glutamine, histidine, and serine;

    v) one or more buffers; and

    vi) one or more anti-foaming agents.

2. The compounded cell culture medium powder formulation of claim 1, wherein the basal medium powder comprises Dulbecco's Modified Eagle's Medium, Ham's Medium F12, Eagle's Minimal Essential Medium, RPMI 1640 Medium, and Dulbecco's Modified Eagle's Medium/Ham's F12 Medium (DMEM/F-12; 1:1 ratio).

3. The compounded cell culture medium powder formulation of claim 1, wherein the basal medium powder comprises one or more of the following components or a combination thereof: biotin, calcium chloride, choline chloride, cyanocobalamin (B12), D+ mannose, D-calcium pantothenate, dextrose (anhydrous), DL-alpha-lipoic acid, ferric nitrate $9H_20$, ferrous sulfate $7H_20$, folic acid, glycine, hypoxanthine, I-inositol, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine HCl H_20, L-cystine 2HCl, L-glutamic acid (anhydrous), L-glutamine, L-glutathione, L-histidine FB, L-histidine HCl, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylaline, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, magnesium chloride, magnesium sulfate anhydrous, niacinamide, O-phosphoryl-ethanolamine, potassium chloride, putrescine 2HCl, pyridoxal HCl, pyridoxine HCl, riboflavin, sodium chloride, sodium phosphate monobasic H_20, sodium

phosphate dibasic anhydrous, sodium pyruvate, thiamine HCl, thymidine, zinc sulfate $7H_20$, cupric sulfate, selenium dioxide, linoleic acid, beta-mercaptoethanol and ethanolamine free-base FB.

4. The compounded cell culture medium powder formulation of claim 3, wherein the basal medium powder has a concentration of 8-30 g/L upon hydration to form a cell culture medium.

5. The compounded cell culture medium powder formulation of claim 4, wherein the basal medium powder has a concentration of about 13 g/L upon hydration to form a cell culture medium.

6. The compounded cell culture medium powder formulation of claims 1-5 further comprising a pH indicator.

7. The compounded cell culture medium powder formulation of claim 6, wherein the pH indicator is Phenol Red Na, wherein the Phenol Red Na is present at a concentration of about 0.001 to about 0.02 g/L upon hydration to form a cell culture medium.

8. The compounded cell culture medium powder formulation of claim 7, wherein the Phenol Red Na is present at a concentration of about 0.0069 g/L upon hydration to form a cell culture medium.

9. The compounded cell culture medium powder formulation of claim 3, wherein the basal medium powder components have the following final concentration upon hydration to form a cell culture medium.

   i) 0.0003-0.003 g/L biotin;

   ii) 0.035-0.33 g/L calcium chloride;

   iii) 0.003-0.03 g/L choline chloride;

   iv) 0.0002-0.002 g/L cyanocobalamin (B12);

   v) 1-10 g/L D+ mannose;

   vi) 0.001-0.01 g/L D-calcium pantothenate;

   vii) 0.3-3.0 g/L dextrose (anhydrous);

viii) 0.00003-0.0003 g/L DL-alpha-lipoic acid;

ix) 0.00001-0.00015 g/L ferric nitrate;

x) 0.00005-0.0015 g/L ferrous sulfate;

xi) 0.001-0.01 g/L folic acid;

xii) 0.007-0.20 g/L glycine;

xiii) 0.001-0.01 g/L hypoxanthine 2Na;

xiv) 0.005-0.05 g/L I-inositol;

xv) 0.003-0.03 g/L L-alanine;

xvi) 0.08-1.4 g/L L-arginine;

xvii) 0.006-0.16 g/L L-asparagine;

xviii) 0.005-0.10 g/L L-aspartic acid;

xix) 0.005-0.05 g/L L-cysteine HCl;

xx) 0.02-0.2 g/L L-cystine 2HCl;

xxi) 0.005-0.15 g/L L-glutamic acid (anhydrous);

xxii) 0.02-1.5 g/L L-glutamine;

xxiii) 0.0003-0.003 g/L L-glutathione;

xxiv) 0.02-0.2 g/L L-histidine HCl;

xxv) 0.03-0.9 g/L L-isoleucine;

xxvi) 0.03-0.9 g/L L-leucine;

xxvii) 0.05-1.5 g/L L-lysine;

xxviii) 0.01-0.3 g/L L-methionine;

xxix) 0.02-0.6 g/L L-phenylaline;

xxx) 0.008-0.25 g/L L-proline;

xxxi) 0.009-0.25 g/L L-serine;

xxxii) 0.03-0.9 g/L L-threonine;

xxxiii) 0.006-0.16 g/L L-tryptophan;

xxxiv) 0.02-0.9 g/L L-tyrosine 2Na;

xxxv) 0.03-0.9 g/L L-valine;

xxxvi) 0.01-0.18 g/L magnesium chloride ;

xxxvii) 0.02-0.12 g/L magnesium sulfate anhydrous;

xxxviii) 0.001-0.01 g/L niacinamide;

xxxix) 0.0005-0.005 g/L O-phoshphoryl-ethanolamine;

xl) 0.1-1.0 g/L potassium chloride;

xli) 0.00002-0.0002 g/L putrescine 2HCl;

xlii) 0.001-0.01 g/L pyridoxal HCl;

xliii) 0.00001-0.0001 g/L pyridoxine HCl,

xliv) 0.0001-0.001 g/L riboflavin,

xlv) 2.0-15 g/L sodium chloride,

xlvi) 0.01-0.2 g/L sodium phosphate monobasic,

xlvii) 0.02-0.2 g/L sodium phosphate dibasic anhydrous,

xlviii) 0.015-0.15 g/L sodium pyruvate,

xlix) 0.001-0.01 g/L thiamine HCl,

l) 0.0001-0.001 g/L thymidine,

li) 0.00006-0.0015 g/L zinc sulfate,

lii) 0.0000003-0.000006 g/L cupric sulfate,

liii) 0.0000005-0.000008 g/L selenium dioxide,

liv) 0.00001-0.0001 g/L linoleic acid,

lv) 0.0001-0.001 g/L beta-mercaptoethanol; and

lvi) 0.0003-0.005 g/L ethanolamine.

10. The compounded cell culture medium powder formulation of claim 9, wherein the basal medium powder components have the following final concentration upon hydration to form a cell culture medium:

i) about 0.001 g/L biotin;

ii) about 0.11665 g/L calcium chloride;

iii) about 0.00998 g/L choline chloride;

iv) about 0.00068 g/L cyanocobalamin (B12);

v) about 3 g/L D+ mannose;

vi) about 0.00312 g/L D-calcium pantothenate;

vii) about 1 g/L dextrose (anhydrous);

viii) about 0.000103 g/L DL-alpha-lipoic acid;

ix) about 0.00005 g/L ferric nitrate $9H_20$;

x) about 0.000417 g/L ferrous sulfate $7H_20$;

xi) about 0.00366 g/L folic acid;

xii) about 0.02626 g/L glycine;

xiii) about 0.0027 g/L hypoxanthine 2Na;

xiv) about 0.01451 g/L I-inositol;

xv) about 0.01336 g/L L-alanine;

xvi) about 0.27435 g/L L-arginine;

xvii) about 0.0225 g/L L-asparagine;

xviii) about 0.01995 g/L L-aspartic acid;

xix) about 0.01756 g/L L-cysteine HCl H_20;

xx) about 0.06256 g/L L-cystine 2HCl;

xxi) about 0.02206 g/L L-glutamic acid (anhydrous);

xxii) about 0.73 g/L L-glutamine;

xxiii) about 0.001 g/L L-glutathione;

xxiv) about 0.07348 g/L L-histidine HCl;

xxv) about 0.1057 g/L L-isoleucine;

xxvi) about 0.11096 g/L L-leucine;

xxvii) about 0.16385 g/L L-lysine;

xxviii) about 0.03224 g/L L-methionine;

xxix) about 0.06748 g/L L-phenylaline;

xxx) about 0.02875 g/L L-proline;

xxxi) about 0.03676 g/L L-serine;

xxxii) about 0.10156 g/L L-threonine;

xxxiii) about 0.01902 g/L L-tryptophan;

xxxiv) about 0.10771 g/L L-tyrosine 2Na $2H_2O$;

xxxv) about 0.09866 g/L L-valine;

xxxvi) about 0.028 g/L magnesium chloride ;

xxxvii) about 0.04884 g/L magnesium sulfate anhydrous;

xxxviii) about 0.00302 g/L niacinamide;

xxxix) about 0.0014 g/L O-phoshphoryl-ethanolamine;

xl) about 0.31182 g/L potassium chloride;

xli) about 0.000081 g/L putrescine 2HCl;

xlii) about 0.003 g/L pyridoxal HCl;

xliii) about 0.000031 g/L pyridoxine HCl,

xliv) about 0.000319 g/L riboflavin,

xlv) about 6.1234 g/L sodium chloride,

xlvi) about 0.0625 g/L sodium phosphate monobasic H_20,

xlvii) about 0.07099 g/L sodium phosphate dibasic anhydrous,

xlviii) about 0.055 sodium pyruvate,

xlix) about 0.00317 g/L thiamine HCl,

l) about 0.000364 g/L thymidine,

li) about 0.000432 g/L zinc sulfate $7H_20$,

lii) about 0.00000125 g/L cupric sulfate $5H_2O$,

liii) about 0.00000222 g/L selenium dioxide,

liv) about 0.000042 g/L linoleic acid,

lv) about 0.00039065 g/L beta-mercaptoethanol; and

lvi) about 0.0012 g/L ethanolamine FB.

11. The compounded cell culture medium powder formulation of claims 1-10, wherein the one or more salts of part i) of claim 1 comprises magnesium chloride in a concentration of 0.5-5 g/L upon hydration to form a cell culture medium.

12. The compounded cell culture medium powder formulation of claims 11, wherein the magnesium chloride has a concentration of about 1.428 g/L upon hydration to form a cell culture medium.

13. The compounded cell culture medium powder formulation of claims 1-12, wherein the growth factor comprises recombinant insulin.

14. The compounded cell culture medium powder formulation of claims 13, wherein the insulin has a concentration of 0.5-15 mg/L upon hydration to form a cell culture medium.

15. The compounded cell culture medium powder formulation of claims 14, wherein the insulin has a concentration of about 3 mg/L upon hydration to form a cell culture medium.

16. The compounded cell culture medium powder formulation of claims 1-15, wherein the one or more inorganic ions comprise trace metals selected ammonium molybdate, chromium potassium sulfate, cupric sulfate, lithium chloride, manganese sulfate, sodium metasilicate and combinations thereof.

17. The compounded cell culture medium powder formulation of claims 16, wherein the ammonium molybdate is ammonium molybdate $4H_2O$; the chromium potassium sulfate is chromium potassium sulfate $12H_2O$, the cupric sulfate is cupric sulfate $5H_2O$, the lithium chloride is lithium chloride (anhydrous), the manganese sulfate is manganese sulfate H_2O and the sodium metasilicate is sodium metasilicate $9H_2O$.

18. The compounded cell culture medium powder formulation of claims 17, wherein the trace metals have the following final concentration upon hydration to form a cell culture medium:

    i) 0.0005-0.01 mg/L of ammonium molybdate $4H_2O$;

    ii) 0.0001-0.01 mg/L of chromium potassium sulfate $12H_2O$;

    iii) 0.001-0.125 mg/L of cupric sulfate $5H_2O$;

    iv) 0.001-0.1 mg/L of lithium chloride (anhydrous);

    v) 0.00004-0.004 mg/L of manganese sulfate H_2O; and

    vi) 0.04-4.2 mg/L of sodium metasilicate $9H_2O$.

19. The compounded cell culture medium powder formulation of claims 1-18, wherein the compounded cell culture medium powder formulation comprises a combination of ammonium molybdate $4H_2O$, chromium potassium sulfate $12H_2O$, cupric sulfate $5H_2O$, lithium chloride (anhydrous), manganese sulfate H_2O, and sodium metasilicate $9H_2O$.

20. The compounded cell culture medium powder formulation of claims 19, wherein the trace metals have the following final concentration upon hydration to form a cell culture medium:

    i) about 0.0037 mg/L of ammonium molybdate $4H_2O$;

    ii) about 0.001 mg/L of chromium potassium sulfate $12H_2O$;

    iii) about 0.0125 mg/L of cupric sulfate $5H_2O$;

    iv) about 0.01 mg/L of lithium chloride (anhydrous);

    v) about 0.000452 mg/L of manganese sulfate H_2O; and

vi) about 0.4263 mg/L of sodium metasilicate $9H_2O$.

21. The compounded cell culture medium powder formulation of claims 1-20, wherein the amino acid supplement comprises a combination of asparagine H_2O, glutamine, histidine, and serine.

22. The compounded cell culture medium powder formulation of claims 1-21, wherein the amino acid supplement has the following final concentration upon hydration to form a cell culture medium:

i) 0.007-0.07 g/L asparagine H_2O;

ii) 0.25-2.5 g/L glutamine;

iii) 0.5-5.0 g/L histidine, free base; and

iv) 0.01-0.1 g/L serine.

23. The compounded cell culture medium powder formulation of claims 1-22, wherein the amino acid supplement has the following final concentration upon hydration to form a cell culture medium:

i) about 0.0225 g/L asparagine H_2O;

ii) about 0.73 g/L glutamine;

iii) about 1.552 g/L histidine; and

iv) about 0.03676 g/L serine.

24. The compounded cell culture medium powder formulation of claims 1-23, wherein the one or more buffers is selected from the group consisting of 3-(N-morpholino)propanesulfonic acid (MOPS) free acid, 3-(N-morpholino)propanesulfonic acid (MOPS) Na, hydroxyethyl piperazineethanesulfonic acid (HEPES) and sodium bicarbonate.

25. The compounded cell culture medium powder formulation of claims 1-24, wherein the formulation comprises a combination of MOPS free acid and MOPS Na.

26. The compounded cell culture medium powder formulation of claims 1-25, wherein the buffers have the following final concentration upon hydration to form a cell culture medium:

    i) 0.3-3 g/L MOPS free acid; and

    ii) 1.0-10 g/L MOPS Na.

27. The compounded cell culture medium powder formulation of claims 1-26, wherein the anti-foaming agent comprises a polyol copolymer based on ethylene oxide and propylene oxide.

28. The compounded cell culture medium powder formulation of claims 1-27, wherein the anti-foaming agent is Pluronic F68.

29. The compounded cell culture medium powder formulation of claims 1-28, wherein the Pluronic F68 has a concentration of 0.1-10 g/L upon hydration to form a cell culture medium.

30. The compounded cell culture medium powder formulation of claims 29, wherein the Pluronic F68 has a concentration of about 1 g/L upon hydration to form a cell culture medium.

31. A method of making the compounded cell culture medium powder formulation of claims 1-30, comprising combining the basal medium powder; magnesium chloride; insulin; one or more trace metals; an amino acid supplement comprising one or more of asparagine; glutamine; histidine; and serine; one or more buffers; and one or more anti-foaming agents.

32. A method of making a cell culture medium for growing mammalian cells, comprising combining the compounded cell culture medium powder formulation of claims 1-30 with water, thereby making a cell culture medium for growing mammalian cells.

33. The method of claim 32, wherein the method further comprises combining a solution comprising $FeSO_4$ $7H_2O$ and a chelating agent.

34. The method of claim 33, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).

35. The method of claim 34, wherein the wherein the FeSO4 7H2O and EDTA have the following final concentration in the cell culture medium:

    i) 0.004-0.04 g/L $FeSO_4$ $7H_2O$; and

    ii) 0.006-0.06 g/L EDTA.

36. The method of claim 35, wherein the wherein the $FeSO_4$ $7H_2O$ and EDTA have the following final concentration in the cell culture medium:

    i) about 0.0138g/L $FeSO_4$ $7H_2O$; and

    ii) about 0.018625g/L EDTA.

37. The method of claims 32-36, further comprising contacting the cell culture medium with mammalian cells.

38. The method of claim 37, wherein the mammalian cells are selected from the group consisting of Chinese Hamster Ovary (CHO) cells, DP12 CHO cells, DG44 CHO cells, Human Embryonic Kidney (HEK) cells, HEK 293 cells, and baby hamster kidney (BHK) cells.

39. The method of claim 38, wherein the mammalian cells recombinantly express a protein of interest.

40. The method of claim 39, wherein the protein of interest is selected from the group consisting of coagulation Factor VIII (FVIII), variants and fragments thereof.

41. The method of claim 40, wherein the FVIII is selected from wild-type FVIII, B-domain deleted FVIII and FVIII conjugated with a biocompatible polymer.

42. The method of claim 41, wherein the biocompatible polymer is polyethylene glycol (PEG).

43. The method of claim 42, wherein the PEG is covalently attached to the polypeptide at one or more of the factor VIII amino acid positions 81, 129, 377, 378, 468, 487, 491, 504, 556, 570, 711, 1648, 1795, 1796, 1803, 1804, 1808, 1810, 1864, 1903, 1911, 2091, 2118 and 2284.

44. The method of claims 38-43, wherein the mammalian cells are BHK cells.