media components such as animal sera and peptone. Chemically undefined nutrients may lead to lot-to-lot variability and animal-derived products may contaminate media with undesirable constituents. Chemically defined cell culture media ("CDM"), which are of a known composition that is consistent lot-to-lot, have been developed to addresses these concerns. Due to the various advantages associated with the use of CDM for protein production, there is an industry wide trend to move away from serum containing and peptone containing processes in favor of processes that utilize CDM. Various CDM have been described in the patent literature, such as in U.S. Patent Nos. 4,767,704; 5,691,202; 6,048,728; 6,900,056; and 7,601,535, and in U.S. Patent Application Publication Nos. 20030087372 and 20110039330. However, chemically undefined media continue to find use in protein production.

[0006] There is a continuing need to provide improved and cost-effective methods of producing proteins (*e.g.*, antibodies) *in vitro* having acceptable product quality attributes. Cell culture media that modulate one or more product quality attributes are desired. Cell culture media, whether chemically undefined or chemically defined, having components that consistently deliver protein products at lower color intensities while maintaining a desired protein concentration (*e.g.*, ≥ 150 mg/mL) would find use in the development of protein products, such as antibodies, *e.g.*, for subcutaneous injection.

BRIEF SUMMARY ~~OF THE INVENTION~~

[0007] Cell culture media compositions that provide a drug product with an acceptable color are described, as are methods of using the media for cell growth (*i.e.*, cell culture) and/or protein production. Media that provide protein-based drug products with an acceptable color while maintaining a desired protein-based drug product concentration (*e.g.*, ≥ 100 mg/mL or ≥ 150 mg/mL) are also provided and may find use in protein production methods, such as for the production of antibodies for subcutaneous injection. The cell culture media as detailed herein may be chemically undefined or CDM. Compositions comprising a medium as provided herein and a polypeptide (*e.g.*, a polypeptide secreted by a host cell into the medium) and/or a cell comprising an isolated nucleic acid encoding a polypeptide are also contemplated. Polypeptides prepared by the methods detailed herein are provided, as are formulations comprising the polypeptides and a carrier (*e.g.*, a pharmaceutically acceptable

carrier). The polypeptide formulations in one embodimentaspect have an acceptable color and maintain a protein-based drug product concentration of at least 100 mg/mL or 150 mg/mL.

[0008] The cell culture media detailed herein generally comprise one or more of the following components in an amount to effect a protein product quality attribute such as color: (a) cystine or cysteine; (b) vitamin B2, (c) vitamin B6 (pyridoxine and/or pyridoxal, which may be provided as the HCl salt), (d) vitamin B9, (e) vitamin B12, (f) an iron source such as iron nitrate, ferric citrate or ferrous sulfate and (g) hydrocortisone. In one variation, a cell culture medium comprises 2 or 3 or 4 or 5 or 6 or each of components (a), (b), (c), (d), (e), (f) and (g). It is understood that a cell culture medium provided herein may contain any combination of components (a), (b), (c), (d), (e), (f) and (g) the same as if each and every combination were specifically and individually listed. In one embodimentaspect, the cell culture media is a CDM. In another embodimentaspect, the cell culture media is chemically undefined.

[0008a] In one aspect, there is provided a cell culture medium comprising:
from about 200 mg/L to about 1200 mg/L cystine;
from about 0.05 mg/L to about 1.0 mg/L vitamin B2;
from about 0.05 mg/L to about 10.0 mg/L vitamin B6;
from about 0.05 mg/L to about 12.0 mg/L vitamin B9; and
from about 0.05 mg/L to about 2.5 mg/L vitamin B12.

In a particular variation, a cell culture medium detailed herein comprises: (a) from about 300 mg/L (in some embodiments, 200 mg/L) to about 1200 mg/L cystine; (b) from about 0.05 mg/L to about 1.0 mg/L vitamin B2; (c) from about 0.05 mg/L to about 10.0 mg/L vitamin B6; (d) from about 0.05 mg/L to about 12.0 mg/L vitamin B9; and (e) from about 0.05 mg/L to about 2.5 mg/L vitamin B12, and where the cell culture medium (a) may in one variation be a CDM and/or (b) may further comprise one or more of the following components: (1) an iron source, such as ferric citrate or ferrous sulfate (which in one embodimentaspect is present at a concentration of from about 2 μM to about 80 μM), and (2) hydrocortisone (which in one embodimentaspect is present at a concentration from about 0.05 μM to about 0.25 μM).

[0008b] In one aspect, there is provided a cell culture medium comprising:
from about 200 mg/L to about 1200 mg/L cystine;
from about 2 μM to about 80 μM ferric citrate; and
from about 0.05 μM to about 0.5 μM hydrocortisone.

In another variation, a cell culture

medium as detailed herein comprises: (a) from about 300 mg/L (in some embodiments, 200 mg/L) to about 1200 mg/L cystine; (b) from about 2 μM to about 80 μM ferric citrate; and (c) from about 0.05 μM to about 0.5 μM hydrocortisone, and where the cell culture medium (1) may in one variation be a CDM and/or (2) may further comprise one or more of the following components: (A) vitamin B2 (which in one embodimentaspect is present at a concentration of from about 0.05 mg/L to about 1.0 mg/L); (B) vitamin B6 (which in one embodimentaspect is present at a concentration of from about 0.05 mg/L to about 10.0 mg/L); (C) vitamin B9 (which in one embodimentaspect is present at a concentration of from about 0.05 mg/L to about 12.0 mg/L); and (D) vitamin B12 (which in one embodimentaspect is present at a concentration of from about 0.05 mg/L to about 2.5 mg/L). For any medium provided herein, in one variation the medium reduces the presence of charge variants (which in one embodimentaspect are acidic charge variants) when used in a

method of producing a polypeptide as compared to charge variants (which in one embodimentaspect are acidic charge variants) obtained when the polypeptide is produced in a different cell culture medium (*e.g.*, a medium that does not comprise the same medium components or does contains the same medium components but in a different amount). In one variation of the compositions and methods provided herein, charge variants (which in one embodimentaspect are acidic charge variants) constitute no more than 25% or 20% or 18% or 15% or 10% of the polypeptide product. In another variation of the compositions and methods provided herein, at least 75% or 80% or 85% or 90% or 95% or more of the polypeptide product is a main species protein. In some variations, the main species protein is a quantitatively predominant protein as identified by the amino acid sequence, the secondary structure, and/or the tertiary structure of the protein. In some variations, the main species protein is a quantitatively predominant protein that is identified by one or more post-translational modifications. In some variations, the post-translational modification is glycosylation. It is understood that a percentage of a polypeptide product as described herein can be determined before purification of the polypeptide product, after purification of the polypeptide product, or at any step during a polypeptide purification process.

[0009] Acidic variants may be evaluated by a variety of methods, but preferably such methods include one, two, three, four, or five of: ion exchange chromatography (IEC) wherein the composition is treated with sialidase before, after, and/or during the IEC (*e.g.* to evaluate sialylated variant), reduced CE-SDS (*e.g.* to evaluate disulfide reduced variant), non-reduced CE-SDS (e.g to evaluate non-reducible variant), boronate chromatography (*e.g.* to evaluate glycated variant), and peptide mapping (*e.g.* to evaluate deamidated variant). In one variation, the overall acidic variants are evaluated by ion exchange chromatography, for example using a weak cation exchanger and/or cation exchanger with carboxylate functional group (for example, using a DIONEX PROPAC™ WCX-10 chromatography column).

[0010] In another embodimentaspect of the inventiondisclosure, the cell culture media detailed herein generally comprise one or more of the following components in an amount to effect a protein product quality attribute such as color: (a) cystine; (b) vitamin B1; (c) vitamin B2; (d) vitamin B3; (e) vitamin B5; (f) vitamin B6 (pyridoxine and/or pyridoxal, which may be provided as the HCl salt); (g) vitamin B7; (h) vitamin B9; (i) vitamin B12; and (j) an iron source such as iron nitrate, ferric citrate or ferrous sulfate. In one variation, a cell culture medium comprises 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or each of components (a), (b), (c), (d), (e), (f), (g), (h), (i), and

(j). It is understood that a cell culture medium provided herein may contain any combination of components (a), (b), (c), (d), (e), (f), (g), (h), (i), and (j) the same as if each and every combination were specifically and individually listed. In one embodimentaspect, the cell culture media is a CDM. In another embodimentaspect, the cell culture media is chemically undefined. In a particular variation, a cell culture medium detailed herein comprises: (a) from about 0.8 mM (in some embodiments, 0.7 mM) to about 2.5 mM cystine; (b) from about 0.11 μM to about 0.72 μM vitamin B2; (c) from about 4.5 μM to about 30.0 μM vitamin B6; (c) from about 3.4 μM to about 22.0 μM vitamin B9; and (d) from about 0.2 μM to about 1.5 μM vitamin B12, and where the cell culture medium (a) may in one variation be a CDM and/or (b) may further comprise one or more of the following components: (1) an iron source, such as ferric citrate or ferrous sulfate (which in one embodimentaspect is present at a concentration of from about 11.0 μM to about 36.0 μM), (2) vitamin B1 (which in one embodimentaspect is present at a concentration from about 2.0 μM to about 14.0 μM), (3) vitamin B3 (which in one embodimentaspect is present at a concentration from about 11.0 μM to about 72.0 μM), (4) vitamin B5 (which in one embodimentaspect is present at a concentration from about 6.8 μM to about 44.0 μM), and (5) vitamin B7 (which in one embodimentaspect is present at a concentration from about 0.02 μM to about 0.24 μM).

[0011] Use of the cell culture media detailed herein may increase the stability (*e.g.*, physical stability and/or chemical stability) of a protein product, such as by reducing oxidation of a main species protein, as compared to protein products produced in a cell culture medium that is of a different composition (*e.g.*, a medium that does not comprise the media components and/or amount of components as detailed herein). Use of the cell culture media detailed herein may also reduce colored forms of a polypeptide product as compared to polypeptide products produced in a medium that is of a different composition (*e.g.*, a medium that does not comprise the media components and/or amount of components as detailed herein). Use of the cell culture media detailed herein may also reduce binding of a polypeptide product to other substances in a cell culture vessel (*e.g.*, adducts) as compared to polypeptide products produced in a medium that is of a different composition (*e.g.*, a medium that does not comprise the media components and/or amount of components as detailed herein). A method of increasing stability of a polypeptide composition is contemplated, as are methods of reducing the presence and/or amount of a colored form of a polypeptide product and reducing the binding of a polypeptide product to other matters in a cell culture vessel such as adducts.

[0012] Methods of preparing a formulation comprising a polypeptide (*e.g.*, an antibody) are also provided, comprising the steps of producing a polypeptide as provided by any method detailed herein and combining the polypeptide with one or more formulation components, such as a pharmaceutically acceptable carrier or excipient.

[0013] A formulation comprising a polypeptide (*e.g.*, an antibody) produced by any method detailed herein are also provided. A formulation may comprise a polypeptide and a pharmaceutically acceptable carrier or excipient. A polypeptide formulation, which may be suitable for administration to an individual, may comprise an isolated and/or purified antibody and have one or more desirable product quality attributes, such as an acceptable color. In one embodimentaspect, a formulation comprising a polypeptide product obtained by any of the methods provided herein comprises the polypeptide at a concentration of at least 100 mg/mL or at least 150 mg/mL. Formulations comprising at least 100 mg/mL or at least 150 mg/mL polypeptide product (*e.g.*, an antibody, such as an IgG1 antibody) may also be of an acceptable color. Such formulations may be suitable for injection, such as subcutaneous injection into an individual, which in one embodimentaspect is a human. In some aspectsembodiments, a polypeptide drug product suitable for injection is at a concentration greater than at least 100 mg/mL, at least 125 mg/mL, or at least 150 mg/mL and has a color intensity value greater than B3, B4, B5, B6, B7, B8, or B9 as measured by the COC assay. It is understood that the color intensity value as determined by the COC assay can be any one of, but not limited to, brown (B), brownish-yellow (BY), yellow (Y), greenish-yellow (GY), or red (R), wherein higher values indicate a lighter color intensity. In some embodimentsaspects, a polypeptide drug product suitable for injection is at a concentration greater than at least 100 mg/mL, at least 125 mg/mL, or at least 150 mg/mL and has a color intensity value less than a color intensity value of a reference solution as measured by a color assay (*e.g.*, the Total Color assay or the NIFTY assay). Formulations as provided herein may comprise a polypeptide product where no more than 25% or 20% or 18% or 15% or 10% of the polypeptide product is a polypeptide charge variant (which in one aspect embodiment is an acidic charge variant). Formulations as detailed herein may also comprise a polypeptide product where at least 75% or 80% or 85% or 90% or 95% or more of the polypeptide product is a main species protein. In a particular variation, a formulation comprising a polypeptide (*e.g.*, an antibody) product is provided where the formulation comprises the polypeptide product at a concentration of greater than 100 mg/mL or greater than 125 mg/mL or greater than 150 mg/mL and where the formulations is of an

acceptable color and where no more than 25% or 20% or 18% or 15% or 10% of the polypeptide product is a polypeptide charge variant (which in one embodimentaspect is an acidic charge variant).

[0014] Compositions comprising the cell culture medium and one or more other components, such as a cell and/or a desired polypeptide (*e.g.*, an antibody), are also provided. The compositions encompass any and all phases of cell culture, such as seeding, cell growth, and cell production/maintenance.

[0014a] In one aspect, there is provided a composition comprising (a) a cell comprising an isolated nucleic acid encoding a polypeptide; and (b) a medium as described herein.

[0014b] In one aspect, there is provided a composition comprising: (a) a polypeptide; and (b) a medium as described herein.

In one variation is provided a composition comprising: (a) a cell comprising an isolated nucleic acid encoding a polypeptide; and (b) a cell culture medium as provided herein. In another variation is provided a composition comprising: (a) a polypeptide; and (b) a cell culture medium as provided herein, where in one embodimentaspect the polypeptide is secreted into the medium by a cell comprising an isolated nucleic acid encoding the polypeptide or is released into the medium by lysis of a cell comprising an isolated nucleic acid encoding the polypeptide. The cell of the composition may be any cell detailed herein (*e.g.*, a CHO cell) and the cell culture medium of the composition may be any medium detailed herein, the same as if each and every combination of cell and medium where specifically and individually listed. Likewise, the polypeptide of the composition may be any polypeptide detailed herein and the medium of the composition may be any medium detailed herein, the same as if each and every combination of polypeptide and medium where specifically and individually listed.

[0015] Methods of growing cells (*i.e.*, culturing cells) by contacting the cells with a cell culture medium as detailed herein are provided.

[0015a] In one aspect, there is provided a method of culturing cells, comprising the step of contacting the cells with a cell culture medium comprising:

from about 200 mg/L to about 1200 mg/L cystine;

from about 0.05 mg/L to about 1.0 mg/L vitamin B2;

from about 0.05 mg/L to about 10.0 mg/L vitamin B6;

from about 0.05 mg/L to about 12.0 mg/L vitamin B9; and

from about 0.05 mg/L to about 2.5 mg/L vitamin B12.

In a particular variation of a method of growing a cell (*i.e.*, culturing a cell), the cell culture medium is a CDM. In one variation, a method of growing a cell (*i.e.*, culturing a cell) comprises the step of contacting the cell with a cell culture medium comprising: (a) from about 300 mg/L (in some embodiments, 200 mg/L) to about 1200 mg/L cystine; (b) from about 0.05 mg/L to about 1.0 mg/L vitamin B2; (c) from about 0.05 mg/L to about 10.0 mg/L vitamin B6; (d) from about 0.05 mg/L to about 12.0 mg/L vitamin B9; and (e) from about 0.05 mg/L to about 2.5 mg/L vitamin B12, where the cell culture medium may further comprise one or more of the following components: (1) an iron source, such as ferric citrate or ferrous sulfate (which in one embodimentaspect is present at a concentration of from about 2 μM to about 80 μM), and (2) hydrocortisone (which in one embodimentaspect is present at a concentration of from about 0.05 μM to about 0.25 μM).

[0015b] In one aspect, there is provided a method of culturing cells, comprising the step of contacting the cells with a cell culture medium comprising:

from about 200 mg/L to about 1200 mg/L cystine;

from about 2 μM to about 80 μM ferric citrate; and

from about 0.05 μM to about 0.5 μM hydrocortisone.

In another variation, a method of growing a cell (*i.e.*, culturing a cell) is provided where the method

comprises the step of contacting the cell with a cell culture medium comprising: (a) from about 300 mg/L (in some embodiments, 200 mg/L) to about 1200 mg/L cystine; (b) from about 2 μM to about 80 μM ferric citrate; and (c) from about 0.05 μM to about 0.5 μM hydrocortisone, where the cell culture medium may further comprise one or more of the following components: (1) vitamin B2 (which in one embodimentaspect is present at a concentration of from about 0.05 mg/L to about 1.0 mg/L); (2) vitamin B6 (which in one embodimentaspect is present at a concentration of from about 0.05 mg/L to about 10.0 mg/L); (3) vitamin B9 (which in one embodimentaspect is present at a concentration of from about 0.05 mg/L to about 12.0 mg/L); and (4) vitamin B12 (which in one embodimentaspect is present at a concentration of from about 0.05 mg/L to about 2.5 mg/L). In any method of growing cells (*i.e.*, culturing cells) provided herein, the cells may be contacted with the cell culture medium during the cells' growth phase and/or production phase. Contacting the cells with the medium detailed herein at any phase of cell culture is contemplated, such as during cell growth, production and maintenance. As is understood by a skilled artisan, cells are contacted with a medium as detailed herein under conditions (*e.g.*, temperature, pH, osmolality, etc.) which promote cell maintenance and/or growth, including production of a polypeptide.

[0016] In another variation of the disclosureinvention, a method of growing a cell (*i.e.*, culturing a cell) comprises the step of contacting the cell with a cell culture medium comprising: (a) from about 0.8 mM (in some embodiments, 0.7 mM) to about 2.5 mM cystine; (b) from about 0.11 μM to about 0.72 μM vitamin B2; (c) from about 4.5 μM to about 30.0 μM vitamin B6; (c) from about 3.4 μM to about 22.0 μM vitamin B9; and (d) from about 0.2 μM to about 1.5 μM vitamin B12, and where the cell culture medium may further comprise one or more of the following components: (1) an iron source, such as ferric citrate or ferrous sulfate (which in one embodimentaspect is present at a concentration of from about 11.0 μM to about 36.0 μM), (2) vitamin B1 (which in one embodimentaspect is present at a concentration from about 2.0 μM to about 14.0 μM), (3) vitamin B3 (which in one embodimentaspect is present at a concentration from about 11.0 μM to about 72.0 μM), (4) vitamin B5 (which in one embodimentaspect is present at a concentration from about 6.8 μM to about 44.0 μM), and (5) vitamin B7 (which in one embodimentaspect is present at a concentration from about 0.02 μM to about 0.24 μM).

[0017] In yet another variation of the disclosureinvention, a method of growing a cell (*i.e.*, culturing a cell) comprises the step of contacting the cell with a cell culture medium comprising from about 0.7 mM to about 2.5 mM cystine; from about 2 μM to about 80 μM ferric citrate; from

about 0.05 μM to about 0.5 μM hydrocortisone; from about 0.11 μM to about 0.72 μM vitamin B2; from about 4.5 μM to about 30.0 μM vitamin B6; from about 3.4 μM to about 22.0 μM vitamin B9; and from about 0.2 μM to about 1.5 μM vitamin B12, and where the cell culture medium may further comprise one or more of the following components: (1) vitamin B1 (which in one embodimentaspect is present at a concentration from about 2.0 μM to about 14.0 μM), (2) vitamin B3 (which in one embodimentaspect is present at a concentration from about 11.0 μM to about 72.0 μM), (3) vitamin B5 (which in one embodimentaspect is present at a concentration from about 6.8 μM to about 44.0 μM), and (4) vitamin B7 (which in one embodimentaspect is present at a concentration from about 0.02 μM to about 0.24 μM).

[0018] Methods of producing a polypeptide by growing in a cell culture medium (*i.e.*, culturing in a cell culture medium) a cell comprising an isolated nucleic acid encoding the polypeptide are also provided, wherein: (a) the cell expresses the polypeptide and (b) the cell culture medium comprises: (1) from about 300 mg/L (in some embodiments, 200 mg/L) to about 1200 mg/L cystine; (2) from about 0.05 mg/L to about 1.0 mg/L vitamin B2; (3) from about 0.05 mg/L to about 10.0 mg/L vitamin B6; (4) from about 0.05 mg/L to about 12.0 mg/L vitamin B9; and (5) from about 0.05 mg/L to about 2.5 mg/L vitamin B12, and where the cell culture medium may further comprise one or more of the following components: (A) an iron source, such as ferric citrate or ferrous sulfate (which in one embodimentaspect is present at a concentration of from about 2 μM to about 80 μM) and (B) hydrocortisone (which in one embodimentaspect is present at a concentration from about 0.05 μM to about 0.25 μM).

[0018a] In one aspect, there is provided a method of producing a polypeptide comprising the step of culturing in a cell culture medium a cell comprising an isolated nucleic acid encoding the polypeptide, wherein:

(a) the cell culture medium comprises:

from about 200 mg/L to about 1200 mg/L cystine;

from about 0.05 mg/L to about 1.0 mg/L vitamin B2;

from about 0.05 mg/L to about 10.0 mg/L vitamin B6;

from about 0.05 mg/L to about 12.0 mg/L vitamin B9;

from about 0.05 mg/L to about 2.5 mg/L vitamin B12; and

(b) the cell expresses the polypeptide.

[0018b] In one aspect, there is provided a method of producing a polypeptide comprising the step of culturing in a cell culture medium a cell comprising an isolated nucleic acid encoding the polypeptide, wherein:

(a) the cell culture medium comprises:

from about 200 mg/L to about 1200 mg/L cystine;

from about 2 μM to about 80 μM ferric citrate; and

from about 0.05 μM to about 0.5 μM hydrocortisone; and

(b) the cell expresses the polypeptide.

In another variation, a method of producing a polypeptide by growing in a cell culture medium (*i.e.*, culturing in a cell culture medium) a cell comprising an isolated nucleic acid encoding the polypeptide is provided, wherein: (a) the cell expresses the polypeptide and (b) the cell culture medium comprises: (1) from about 300 mg/L (in some embodiments, 200 mg/L) to about 1200 mg/L cystine; (2) from about 2 μM to about 80 μM ferric citrate; and (3) from about 0.05 μM to about 0.5 μM hydrocortisone, where the cell culture medium may further comprise one or more of the following components: (A) vitamin B2 (which in one embodimentaspect is present at a concentration of from about 0.05 mg/L to about 1.0 mg/L); (B) vitamin B6 (which in one embodimentaspect is present at a concentration of from about 0.05 mg/L to about 10.0 mg/L); (C) vitamin B9 (which in one embodimentaspect is present at a concentration of from about 0.05 mg/L to about 12.0 mg/L); and (D) vitamin B12 (which in one embodimentaspect is present at a concentration of from about 0.05 mg/L to about 2.5 mg/L).

[0019] In another variation of the disclosure~~invention~~, a method of producing a polypeptide by growing in a cell culture medium (*i.e.*, culturing in a cell culture medium) a cell comprising an isolated nucleic acid encoding the polypeptide are also provided, wherein: (a) the cell expresses the polypeptide and (b) the cell culture medium comprises: (a) from about 0.8 mM (in some embodiments, 0.7 mM) to about 2.5 mM cystine; (b) from about 0.11 μM to about 0.72 μM vitamin B2; (c) from about 4.5 μM to about 30.0 μM vitamin B6; (c) from about 3.4 μM to about 22.0 μM vitamin B9; and (d) from about 0.2 μM to about 1.5 μM vitamin B12, and where the cell culture medium may further comprise one or more of the following components: (1) an iron source, such as ferric citrate or ferrous sulfate (which in one embodiment~~aspect~~ is present at a concentration of from about 11.0 μM to about 36.0 μM), (2) vitamin B1 (which in one embodiment~~aspect~~ is present at a concentration from about 2.0 μM to about 14.0 μM), (3) vitamin B3 (which in one embodiment~~aspect~~ is present at a concentration from about 11.0 μM to about 72.0 μM), (4) vitamin B5 (which in one embodiment~~aspect~~ is present at a concentration from about 6.8 μM to about 44.0 μM), and (5) vitamin B7 (which in one embodiment~~aspect~~ is present at a concentration from about 0.02 μM to about 0.24 μM).

[0020] In yet another variation of the disclosure~~invention~~, a method of producing a polypeptide by growing in a cell culture medium (*i.e.*, culturing in a cell culture medium) a cell comprising an isolated nucleic acid encoding the polypeptide are also provided, wherein: (a) the cell expresses the polypeptide and (b) the cell culture medium comprises: from about 0.7 mM to about 2.5 mM cystine; from about 2 μM to about 80 μM ferric citrate; from about 0.05 μM to about 0.5 μM hydrocortisone; from about 0.11 μM to about 0.72 μM vitamin B2; from about 4.5 μM to about 30.0 μM vitamin B6; from about 3.4 μM to about 22.0 μM vitamin B9; and from about 0.2 μM to about 1.5 μM vitamin B12, and where the cell culture medium may further comprise one or more of the following components: (1) vitamin B1 (which in one embodiment~~aspect~~ is present at a concentration from about 2.0 μM to about 14.0 μM), (2) vitamin B3 (which in one embodiment~~aspect~~ is present at a concentration from about 11.0 μM to about 72.0 μM), (3) vitamin B5 (which in one embodiment~~aspect~~ is present at a concentration from about 6.8 μM to about 44.0 μM), and (4) vitamin B7 (which in one embodiment~~aspect~~ is present at a concentration from about 0.02 μM to about 0.24 μM).

[0020a] In one aspect, there is provided a polypeptide produced by the method as described herein.

[0021] The polypeptide produced by a method or present in a composition described herein may in one variation be an antibody, such as an IgG1 antibody. In one embodiment~~aspect~~, the polypeptide produced by a method or present in a composition described herein is an anti-

VEGF, anti-mesothelin, anti-PCSK9 or anti-Beta7 antibody. Polypeptides produced according to the methods provided herein or present in the described compositions may be isolated from the cell culture medium and may be further purified. Polypeptides (such as antibodies) may also be concentrated to achieve a desirable concentration. Methods of concentrating polypeptides are known in the field, for example, the concentration of a polypeptide or protein may be increased by using ultrafiltration. In a particular variation, the polypeptide is isolated at a concentration of at least 100 mg/mL or 150 mg/mL and/or appears as a colorless or slightly colored liquid. In a particular variation, a composition comprises the isolated polypeptide at a concentration of at least 100 mg/mL and/or appears as a colorless or slightly colored liquid. In another variation, a composition comprises the isolated polypeptide at a concentration of at least 1 mg/mL or 10 mg/mL or 50 mg/mL or 75 mg/mL and/or appears as a colorless or slightly colored liquid. In another variation, a composition comprises the isolated polypeptide at a concentration of at least about any one of 1 mg/mL or 10 mg/mL or 50 mg/mL or 75 mg/mL and/or appears as a colorless or slightly colored liquid. In another variation, a composition comprises the isolated polypeptide at a concentration of at least about any one of 1 mg/mL or 10 mg/mL or 50 mg/mL or 75 mg/mL to about 125 mg/mL or to about 150 mg/mL and/or appears as a colorless or slightly colored liquid.

[0021a] In one aspect, there is provided a pharmaceutical composition comprising a polypeptide as described herein and a pharmaceutically acceptable carrier.

Also

described is a composition comprising a polypeptide obtained by a method detailed herein and a pharmaceutically acceptable carrier.

[0021b] In one aspect, there is provided a kit for supplementing a cell culture medium with chemically defined constituents, the kit comprising:

cystine in an amount to provide from about 200 mg/L to about 1200 mg/L cystine in the cell culture medium;

vitamin B2 in an amount to provide from about 0.05 mg/L to about 1.0 mg/L vitamin B2 in the cell culture medium;

vitamin B6 in an amount to provide from about 0.05 mg/L to about 10.0 mg/L vitamin B6 in the cell culture medium;

vitamin B9 in an amount to provide from about 0.05 mg/L to about 12.0 mg/L vitamin B9 in

the cell culture medium; and

vitamin B12 in an amount to provide from about 0.05 mg/L to about 2.5 mg/L vitamin B12

[0022] A kit for supplementing a cell culture medium with chemically defined constituents is also described, the kit comprising: (a) cystine in an amount to provide from about 300 mg/L (in some embodiments, 200 mg/L) to about 1200 mg/L cystine in the cell culture medium; (b) vitamin B2 in an amount to provide from about 0.05 mg/L to about 1.0 mg/L vitamin B2 in the cell culture medium; (c) vitamin B6 in an amount to provide from about 0.05 mg/L to about 10.0 mg/L vitamin B6 in the cell culture medium; (d) vitamin B9 in an amount to provide from about 0.05 mg/L to about 12.0 mg/L vitamin B9 in the cell culture medium; and (e) vitamin B12 in an amount to provide from about 0.05 mg/L to about 2.5 mg/L vitamin B12 in the cell culture medium, where the kit may further comprise one or more of the following components (1) an iron source, such as ferric citrate or ferrous sulfate (which in one embodiment~~aspect~~ is in an amount to provide the iron source at a concentration of from about 2 μM to about 80 μM) and (2) hydrocortisone (which in one embodiment~~aspect~~ is in an amount to provide hydrocortisone at a concentration from about 0.05 μM to about 0.25 μM).

[0022a] In one aspect, there is provided a kit for supplementing a cell culture medium with chemically defined constituents, the kit comprising:

cystine in an amount to provide from about 200 mg/L to about 1200 mg/L cystine in the cell culture medium;

ferric citrate in an amount to provide a concentration of from about 2 μM to about 80 μM ferric citrate in the cell culture medium; and

hydrocortisone in an amount to provide a concentration of from about 0.05 μM to about 0.5 μM hydrocortisone in the cell culture medium.

In another

variation, a kit for supplementing a cell culture medium with chemically defined constituents is described, the kit comprising: (a) cystine in an amount to provide from about 300 mg/L (in some embodiments, 200 mg/L) to about 1200 mg/L cystine in the cell culture medium; (b) ferric citrate in an amount to provide a concentration of from about 2 μM to about 80 μM ferric citrate in the cell culture medium; and (c) hydrocortisone in an amount to provide a concentration of from about 0.05 μM to about 0.5 μM hydrocortisone in the cell growth (*i.e.*, cell culture) medium, where the kit may further comprise one or more of the following components: (1) vitamin B2 (which in one embodimentaspect is in an amount to provide from about 0.05 mg/L to about 1.0 mg/L of vitamin B2 in the cell culture medium); (2) vitamin B6 (which in one embodimentaspect is in an amount to provide from about 0.05 mg/L to about 10.0 mg/L of vitamin B6 in the cell culture medium); (3) vitamin B9 (which in one embodimentaspect is in an amount to provide from about 0.05 mg/L to about 12.0 mg/L of vitamin B9 in the cell culture medium); and (4) vitamin B12 (which in one embodimentaspect is in an amount to provide from about 0.05 mg/L to about 2.5 mg/L of vitamin B12 in the cell culture medium). Also provide herein is a kit for supplementing a cell culture medium with chemically defined constituents, wherein the kit comprises: (a) cystine in an amount to provide from about 0.8 mM (in some embodiments, 0.7 mM) to about 2.5 mM cystine in the cell culture medium; (b) vitamin B2 in an amount to provide from about 0.11 μM to about 0.72 μM vitamin B2 in the cell culture medium; (c) vitamin B6 in an amount to provide from about 4.5 μM to about 30.0 μM vitamin B6 in the cell culture medium; (d) vitamin B9 in an amount to provide from about 3.4 μM to about 22.0 μM vitamin B9 in the cell culture medium and (e) vitamin B12 in an amount to provide from about 0.2 μM to about 1.5 μM vitamin B12 in the cell culture medium, and where the kit may further comprise one or more of the following components: (1) an iron source, such as ferric citrate or ferrous sulfate (which in one embodimentaspect is in an amount to provide from about 11.0 μM to about 36.0 μM in the cell culture medium), (2) vitamin B1 (which in one embodimentaspect is in an amount to provide from about 2.0 μM to about 14.0 μM in a cell culture medium), (3) vitamin B3 (which in one embodimentaspect is in an amount to provide from about 11.0 μM to about 72.0 μM in a cell culture medium), (4) vitamin B5 (which in one embodimentaspect is in an amount to provide from about 6.8 μM to about 44.0 μM in a cell culture medium), and (5) vitamin B7 (which in one embodimentaspect is in an amount to provide from about 0.02 μM to about 0.24 μM in a cell culture medium). Kits may also comprise instructions for use, such as instructions for preparing a