forms. For example, iron, while an essential cofactor for many cellular functions, can be toxic to cells. In undefined media, biological chelators such as transferrin are relied upon to deliver iron without toxicity (Bjare U. Serum-free cell culture. *Pharmacology & Therapeutics.* 1992;53(3):355-374, which is incorporated by reference herein in its entirety). In CDM, non-protein chelators such as citrate must be studied and implemented to prevent iron toxicity. CDM typically contain a large number of components, which can interact in various manners that not necessarily readily apparent. Successful implementation of CDM requires understanding how the components chemically interact and how to keep them in biologically available forms. Understanding about how the components interact is therefore also important in obtaining optimal media and supplements that comprise undefined media.

[0006] There is accordingly a need for improved methods of preparing cell culture media, feeds and supplements, to reduce potential issues due to interactions between components.

SUMMARY OF THE DISCLOSURE

[0007] We have identified that in cell culture media, feeds, or additives comprising cysteine, cysteine oxidation to cystine readily occurs in the presence of trace metals catalysts, primarily copper and iron. When copper catalyzes cysteine oxidation it is converted to an insoluble copper (I) form. Once there is no cysteine left to be oxidized, the copper usually returns to a soluble form. If, however, the solution goes through sterile filtration while cysteine oxidation is occurring, at least a proportion of the copper will typically be in an insoluble form and will be removed from the media, reducing the level of copper in the medium. This is undesirable, as copper is a trace metal that is required in many cell cultures, so the loss of copper from the solution means that the resulting media will provide less copper to any cultured cells that what was intended. This will typically result in lower productivity. An object of the present disclosure is therefore to minimize and/or avoid loss of copper from solutions (such as cell culture medium, cell culture feed or cell culture additive) during filtration.

[0008] In one aspect, the present disclosure provides a method of preventing loss of copper in a solution, wherein the solution is a cell culture medium, cell culture feed or cell culture additive comprising copper (II) and cysteine, wherein the method comprises having substantially the same amount of soluble copper before and after filtering the solution, wherein the filtering is performed with the solution comprising a dissolved oxygen (dO_2) level of at least 6% dO_2, optionally a dissolved oxygen (dO_2) level of at least 8% dO_2 and wherein the filtering is performed on a volume of at least 50 L. In a first example the present disclosure provides a method of preventing loss of copper in a solution. The solution is a cell culture medium, cell culture feed or cell culture additive comprising copper (II) and cysteine. The method comprises having substantially the same amount of soluble copper before and after filtering the solution.

[0009] In an example, the filtering is performed with the solution comprising a dissolved oxygen (dO_2) at at least a specified level. Maintaining the level of oxygen at at

(EDTA), diethylenetriamine pentaacetic acid (DTPA), neocuprione, bathocuprione, D-penicillamine, triethylenetetramine (TETA), dimercaprol (BAL), 8-hydroxyquinoline, clioquinol, and 5,7-Dichloro-2-[(dimethylamino)methyl]-8-quinolinol (PBT2).

[0035] In one aspect, the present disclosure provides a method of preparing a cell culture medium, cell culture feed or cell culture additive comprising copper (II), cysteine, and other components, without loss of soluble copper, comprising: providing an aqueous solution comprising cysteine and other components; providing an aqueous solution comprising copper (II); separately filtering the aqueous solution comprising cysteine and other components, and the aqueous solution comprising copper (II), wherein the filtering is performed with the solution comprising a dissolved oxygen (dO_2) level of at least 6% dO_2, optionally a dissolved oxygen (dO_2) level of at least 8% dO_2 and wherein the filtering is performed on a volume of at least 50 L; and combining the filtered solutions to provide the cell culture medium, cell culture feed or cell culture additive. In a second example, the disclosure provides a method of preparing a cell culture medium, cell culture feed or cell culture additive comprising copper (II), cysteine, and other components, without loss of soluble copper. The method comprises providing an aqueous solution comprising cysteine and other components; providing an aqueous solution comprising copper (II); separately filtering the aqueous solution comprising cysteine and other components, and the aqueous solution comprising copper (II); and combining the filtered solutions to provide the cell culture medium, cell culture feed or cell culture additive.

[0036] Preparation of the aqueous solution comprising cysteine and other components may comprise dissolving and/or solubilizing cysteine and other components in an aqueous solvent system (e.g. water). Preparation of the solution comprising cysteine and other components may comprise dissolving and/or solubilizing cysteine and other components in an initial solvent system comprising acid, base, or water miscible organic solvent, followed by dilution of the initial solvent system with water and optionally other components to form the aqueous solution.

[0037] Preparation of the aqueous solution comprising copper (II) may comprise dissolving a copper (II) salt in an aqueous solvent system (e.g. water). Preparation of the solution comprising copper (II) may comprise dissolving and/or solubilizing a copper (II) salt in an initial solvent system comprising acid, base, or water miscible organic solvent, followed by dilution of the initial solvent system with water and optionally other components to form the aqueous solution.

[0038] The acid may be an inorganic or organic acid. Exemplary inorganic acids include hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like. Exemplary organic acids include relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Exemplary bases include ammonia; hydroxides of sodium, potassium, calcium, ammonium, organic amino, or magnesium and the like; carbonates or bicarbonates of sodium, potassium, or ammonium. Exemplary water miscible organic solvents include ethanol, methanol, acetone, dimethylsulfoxide (DMSO) and dimethylformadine.

[0048] The cysteine may be converted substantially into a cysteine derivative that does not comprise a sulfhydryl group prior to adding the copper (II) to the solution. The cysteine derivative may comprise a disulfide and/or a thiazolidine moiety.

[0049] The cysteine derivative may comprises a disulfide. For example, the cysteine derivative may comprise cystine, S-sulfocysteine, S-sulfocysteinylglycine, L-cysteine mixed disulfides, and/or S-sulfoglutathione. The cysteine derivative may comprise cystine.

[0050] The cysteine derivative may comprise a thiazolidine moiety. For example, the cysteine derivative may comprise 4-carboxy-2-methylthiazolidine-2-carboxylate, 2-methyl-1,3-thiazolidine-2,4-dicarboxylic acid, 2-(2-carboxyethyl)21,3-thiazolidine-2,4-dicarboxylic acid, L-2-oxothiazolidine-4-carboxylic acid, 2-alkyl-thiazolidine-4(R)-carboxylic acid, 2-aryl-thiazolidine-4(R)-carboxylic acid and carbohydrate based thiazolidines (e.g. D-ribose-L-cysteine). The cysteine derivative may comprise 4-carboxy-2-methylthiazolidine-2-carboxylate.

[0051] The cysteine derivative may comprise at least one of cystine, 4-carboxy-2-methylthiazolidine-2-carboxylate, S-sulfocysteine, S-sulfocysteinylglycine, cysteine S-linked N-acetyl glucosamine (GlcNAC-cys), homocystine, L-cysteine mixed disulfides, L-cysteine mixed peptides, S-alkylated cysteine, cysteine with a thiol-protecting group (e.g. FMOC-protected cysteine), 2-methyl-1,3-thiazolidine-2, 4-dicarboxylic acid, 2-(2-carboxyethyl)21,3-thiazolidine-2, 4-dicarboxylic acid, reduced and oxidized glutathione (GSH), S-sulfoglutathione, S-acyl-GSH, S-carboxy-L-cysteine, L-2-oxothiazolidine-4-carboxylic acid, 2-alkyl-thiazolidine-4(R)-carboxylic acid, 2-aryl-thiazolidine-4(R)-carboxylic acid, or carbohydrate based thiazolidines (e.g. D-ribose-L-cysteine).

[0052] In examples, the copper may be in the form of a copper (II) chelate. The chelate may comprise at least one chelator selected from ethylenediaminetetraacetic acid (EDTA), diethylenetriamine pentaacetic acid (DTPA), neocuprione, bathocuprione, D-penicillamine, triethylenetetramine (TETA), dimercaprol (BAL), 8-hydroxyquinoline, clioquinol, and 5,7-Dichloro-2-[(dimethylamino)methyl]-8-quinolinol (PBT2).

[0053] In one aspect, the present disclosure provides a method of preparing a cell culture medium, cell culture feed or cell culture additive comprising copper (II), cysteine, and other components, without loss of soluble copper, comprising: filtering a solution comprising the copper (II), cysteine, and other components to provide the cell culture medium, cell culture feed or cell culture additive, wherein the filtering is performed with the solution comprising a dissolved oxygen (dO_2) level of at least 6% dO_2, optionally a dissolved oxygen (dO_2) level of at least 8% dO_2 and wherein the filtering is performed on a volume of at least 50 L. In third example, the disclosure provides a method of preparing a cell culture medium, cell culture feed or cell culture additive comprising copper (II), cysteine, and other components, without loss of soluble copper. The method comprises filtering a solution comprising the copper (II), cysteine, and other components to provide the cell culture medium, cell culture feed or cell culture additive.

[0054] The filtering may be completed within about 24 hours of adding copper (II) to the solution. The filtering may be completed within about 18 hours of adding copper (II) to the

[0084] The at least one (essential) fatty acid (e.g. at least two fatty acids) may be selected from n-3, n-6, n-9 fatty acids and cholesterol. The at least one (essential) fatty acid (e.g. at least two fatty acids) may be selected from linoleic acid (ALA), linolenic acid (LA), doscosahexenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA). For example, the at least one essential fatty acid (e.g. at least two fatty acids) may be selected from ALA and linolenic acid LA.

[0085] The at least one antioxidant (e.g. two or more antioxidants) may comprise at least one of ascorbic acid, taurine, hypotaurine, a tocopherol, and a tocotrienol.

[0086] The at least one corticosteroid (e.g. two or more corticosteroids) may comprise at least one glucocorticoid (e.g. hydrocortisone) or mineralocorticoid (e.g. aldosterone).

[0087] The at least one metal chelator (e.g. two or more chelators) may comprise at least one of ethylenediaminetetraacetic acid (EDTA), diethylenetriamine pentaacetic acid (DTPA), neocuprione, bathocuprione, D-penicillamine, triethylenetetramine (TETA), dimercaprol (BAL), 8-hydroxyquinoline, clioquinol, and 5,7-Dichloro-2-[(dimethylamino)methyl]-8-quinolinol (PBT2), 1,2- bis(2-Aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), 1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis(acetoxymethyl ester) (BAPTA-AM), deferoxamine mesylate, .

[0088] In one aspect, the present disclosure provides a method of culturing a cell in a medium, the method comprising: preventing loss of copper in the medium according to the method as disclosed herein, or preparing the medium according to the method as disclosed herein, contacting the cell with the medium, and culturing the cell in the medium. In a fourth example, the disclosure provides a method of culturing a cell in a medium. The method comprises preventing loss of copper in the medium according to a method of the first example, or preparing the medium according to a method of the second or third example; contacting the cell with the medium; and culturing the cell in the medium.

[0089] The cell may be a eukaryotic, a prokaryotic or an archaea cell. The eukaryotic cell may be a mammalian cell. The cell may be selected from hybridoma cells, CHO cells, COS cells, VERO cells, HeLa cells, HEK 293 cells, PER-C6 cells, K562 cells, MOLT-4 cells, Ml cells, NS-1 cells, COS-7 cells, MDBK cells, MDCK cells, MRC-5 cells, WI-38 cells, WEHI cells, SP2/0 cells, BHK cells (including BHK-21 cells) and derivatives thereof.

[0090] The culturing the cell in the medium may comprise multiplying the cell. The method may further comprise isolating and/or purifying the multiplied cell.

[0091] In one aspect, the disclosure provides a method of making a biological product, comprising: preventing loss of copper in the medium according to the method as disclosed herein or preparing a medium according to the method as disclosed herein, contacting a cell configured to express said biological product with the medium, and culturing the cell in the medium under conditions such that the cell expresses the biological product. In a fifth example, the disclosure provides a method of making a biological product. The method comprises preventing loss of copper in the medium according to the first example or preparing a medium according to the second or third example; contacting a cell configured to express said biological product with the medium; and culturing the cell in the medium under conditions such that the cell expresses the biological product.

[0092] The biological product may be a polypeptide, protein, peptide, hormone, virus or virus like particle, nucleic acid or a fragment thereof; optionally an antibody, or a biologically functional fragment of an antibody.

[0093] The cell may be a eukaryotic, a prokaryotic or an archaea cell. The eukaryotic cell may be a mammalian cell. The cell may be selected from hybridoma cells, CHO cells, COS cells, VERO cells, HeLa cells, HEK 293 cells, PER-C6 cells, K562 cells, MOLT-4 cells, Ml cells, NS-1 cells, COS-7 cells, MDBK cells, MDCK cells, MRC-5 cells, WI-38 cells, WEHI cells, SP2/0 cells, BHK cells (including BHK-21 cells) and derivatives thereof.

[0094] The method may further comprise isolating and/or purifying the product.

[0095] In a sixth example, the method provides a cell culture medium, cell culture feed, or cell culture additive obtainable or obtained by a method of the second or third example.

[0096] In a seventh example, the disclosure provides use of a cell culture medium, cell culture feed, or cell culture additive of the sixth example for culturing cells. The culturing may comprise producing a biological product from said cells. The culturing may comprise multiplying said cells. The culturing may comprise multiplying said cells and producing a biological product from said cells. The use may further comprise isolating the biological product.

BRIEF DESCRIPTION OF THE DRAWINGS

[0097] Embodiments of the invention are further described hereinafter with reference to the accompanying drawings, in which:

Figure 1 illustrates a time course of copper removal by filtration in Genentech Essential Medium. Aliquots (n=3) were filtered at each time-point and the copper content was compared to unfiltered media.

Figure 2 provides an illustration of how several parameters varied over time in exemplary media after trace metals addition. a) provides the concentration profile for cysteine monomer, b) provides the profile for dissolved oxygen (dO_2) concentration compared to % saturation, c) provides the redox potential, and d) provides an overlay of the results for copper removal, cysteine monomer, and dissolved oxygen, allowing comparison of the relative levels of each of these parameters.

Figure 3 illustrates copper removal extended by a) increasing the starting cysteine concentration in an unmixed system b) increasing the starting cysteine concentration in a mixed system c) sparging with nitrogen after cysteine depletion d) sparging with nitrogen prior to cysteine depletion.

WHAT IS CLAIMED IS:

1. A method of preventing loss of copper in a solution,

wherein the solution is a cell culture medium, cell culture feed or cell culture additive comprising copper (II) and cysteine,

wherein the method comprises having substantially the same amount of soluble copper before and after filtering the solution,

wherein the filtering is performed with the solution comprising a dissolved oxygen (dO_2) level of at least 6% dO_2, optionally a dissolved oxygen (dO_2) level of at least 8% dO_2 and wherein the filtering is performed on a volume of at least 50 L.

2. The method of claim 1, further comprising maintaining the dO_2 level of the solution at at least 6% dO_2 (or at least 8%dO_2) for at least 10 minutes prior to commencing the filtering.

3. The method of claim 1 or claim 2, wherein the dO_2 levels are maintained at the required level by:

   a. oxygenating the solution; and/or

   b. optimizing the filtering, wherein said optimizing the filtering comprises increasing filtration rate, filter size and/or filter capacity, such that dO_2 levels do not fall below 6% during filtering.

4. The method of claim 3, wherein the oxygenating comprises:
sparging the solution with a gas comprising oxygen; and/or
agitating or mixing the solution to increase contact of the solution with gas comprising oxygen.

5. The method of claim 4, wherein the gas comprising oxygen is atmosphere.

6. The method of any preceding claim, wherein the filtering is completed within 24 hours of adding copper (II) to the solution.

7. The method of any of claims 1-4, wherein the filtering is performed within 5 hours, 1 hour, or 30 minutes of adding copper (II) to the solution.

8. The method of any preceding claim, wherein the filtering is performed at a rate of at least 0.5% of the total volume per minute, optionally at least 5% of the total volume per minute.

9. The method of any preceding claim, wherein the method comprises providing a cysteine solution and a copper (II) solution; and

the filtering comprises separately filtering the cysteine solution and the copper (II) solution, and then combining the filtered cysteine solution and the filtered copper (II) solution to form the cell culture medium, cell culture feed or cell culture additive.

10. The method of claim 9, wherein the cysteine solution comprises at least the majority of the components of the cell culture medium, cell culture feed or cell culture additive, other than copper.

11. The method of claim 9 or claim 10, wherein the cysteine solution comprises substantially all of the components of the cell culture medium, cell culture feed or cell culture additive other than copper and optionally iron.

12. The method of any of claims 9 to 11, wherein the concentration of copper in the copper (II) solution is in the range of 0.005 µM to 1 M.

13. The method of any preceding claim, wherein the filtering comprises sterile filtering.

14. The method of claim 13, wherein the sterile filtering comprises use of a filtration medium having a pore size of not more than 0.5 µm, optionally not more than 0.2 µm.

15. The method of claim 13 or claim 14, wherein the sterile filtering comprises use of a filtration medium having a pore size of at least 0.1 µm.

16. The method of any preceding claim, wherein the cysteine is provided substantially as a cysteine derivative that does not comprise a sulfhydryl group.

17. The method of any preceding claim, wherein the cysteine is converted substantially into a cysteine derivative that does not comprise a sulfhydryl group prior to adding the copper (II) to the solution.

18. The method of claim 16 or claim 17, wherein the cysteine derivative comprises a disulfide or a thiazolidine moiety.

19. The method of any of claims 16 to 18, wherein the cysteine derivative comprises a disulfide.

20. The method of any of claims 16 to 19, wherein the cysteine derivative comprises a thiazolidine moiety.

21. The method of any of claims 16 to 20, wherein the cysteine derivative is selected from a group consisting of cystine, 4-carboxy-2-methylthiazolidine-2-carboxylate, S-sulfocysteine, S-sulfocysteinylglycine, cysteine S-linked N-acetyl glucosamine (GlcNAC-cys), homocystine, L-cysteine mixed disulfides, L-cysteine mixed peptides, S-alkylated cysteine, cysteine with a thiol-protecting group (e.g FMOC-protected cysteine), 2-methyl-1,3-thiazolidine-2, 4-dicarboxylic acid, 2-(2-carboxyethyl)21,3-thiazolidine-2, 4-dicarboxylic acid, reduced and oxidized glutathione (GSH), S-sulfoglutathione, S-acyl-GSH, S-carboxy-L-cysteine, L-2-oxothiazolidine-4-carboxylic acid, 2-alkyl-thiazolidine-4(R)-carboxylic acid, 2-aryl-thiazolidine-4(R)-carboxylic acid and carbohydrate based thiazolidines (e.g. D-ribose-L-cysteine).

22. The method of claim 21, wherein the cysteine derivative comprises cystine.

23. The method of claim 22, wherein the cysteine derivative comprises 4-carboxy-2-methylthiazolidine-2-carboxylate.

24. The method of any preceding claim, wherein the copper is in the form of a copper (II) chelate.

25. The method of claim 24, wherein the chelate comprises a chelator selected from ethylenediaminetetraacetic acid (EDTA), diethylenetriamine pentaacetic acid (DTPA), neocuprione, bathocuprione, D-penicillamine, triethylenetetramine (TETA), dimercaprol (BAL), 8-hydroxyquinoline, clioquinol, and 5,7-Dichloro-2-[(dimethylamino)methyl]-8-quinolinol (PBT2).

26. A method of preparing a cell culture medium, cell culture feed or cell culture additive comprising copper (II), cysteine, and other components, without loss of soluble copper, comprising:

providing an aqueous solution comprising cysteine and other components;

providing an aqueous solution comprising copper (II);

separately filtering the aqueous solution comprising cysteine and other components, and the aqueous solution comprising copper (II), wherein the filtering is performed with the solution comprising a dissolved oxygen (dO_2) level of at least 6% dO_2, optionally a dissolved oxygen (dO_2) level of at least 8% dO_2 and wherein the filtering is performed on a volume of at least 50 L; and

combining the filtered solutions to provide the cell culture medium, cell culture feed or cell culture additive.

27. The method of claim 26, wherein preparation of the aqueous solution comprising cysteine and other components comprises dissolving and/or solubilizing cysteine and other components in water.

28. The method of claim 26 or claim 27, wherein preparation of the aqueous solution comprising copper (II) comprises dissolving a copper (II) salt in water.

29. The method of any of claims 26 to 28, wherein the concentration of copper in the copper (II) solution is in the range of 0.005 µM to 1 M.

30. A method of preparing a cell culture medium, cell culture feed or cell culture additive comprising copper (II), cysteine, and other components, without loss of soluble copper, comprising:

filtering a solution comprising the copper (II), cysteine, and other components to provide the cell culture medium, cell culture feed or cell culture additive,

wherein the filtering is performed with the solution comprising a dissolved oxygen (dO_2) level of at least 6% dO_2, optionally a dissolved oxygen (dO_2) level of at least 8% dO_2 and wherein the filtering is performed on a volume of at least 50 L.

31. The method of claim 30, wherein the filtering is completed within 24 hours of adding the copper (II) to the solution;

optionally wherein the filtering is performed within 30 minutes of adding copper (II) to the solution.

32. The method of claim 30 or claim 31, wherein the solution is prepared by dissolving and/or solubilizing a copper (II) salt, cysteine and other components in water.

33. The method of any of claims 26 to 32, wherein the each filtering comprises sterile filtering.

34. The method of claim 33, wherein the ~~or~~ each sterile filtering comprises use of a filtration medium having a pore size of not more than 0.5 µm, optionally of not more than 0.2 µm.

35. The method of claim 33 or claim 34, wherein the sterile filtering comprises use of a filtration medium having a pore size of at least 0.1 µm.

36. The method of any of claims 26 to 35, further comprising the features of any of claims 2 to 5 and 13 to 25.

37. The method of any of claims 26 to 36, wherein the other components comprise at least one carbon source, and/or at least one nitrogen source, and/or at least one

vitamin, and/or at least one buffering agent, and/or at least one inorganic salt, and/or at least one trace metal other than copper, and/or at least one polyamine, and/or at least one (essential) fatty acid.

38. The method of claim 37, wherein the at least one carbon source comprises a sugar, optionally a monosaccharide or disaccharide, e.g. glucose and/or deoxyribose.

39. The method of claim 37 or 38, wherein the at least one nitrogen source comprises at least one amino acid; optionally selected from L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cystine, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine, or derivatives thereof.

40. The method of any of claims 37 to 39, wherein the at least one vitamin is selected from ascorbic acid, biotin, choline, D-Ca^{2+}-pantothenate, folic acid, inositol, menadione, niacinamide, nicotinic acid, paraaminobenzoic acid (PABA), pyridoxal, pyridoxine, riboflavin, thiamine, vitamin A acetate, vitamin B_{12} and vitamin D_2.

41. The method of any of claims 37 to 40, wherein the at least one buffering agent comprises carbonate, bicarbonate, phosphate, hydrogen phosphate, dihydrogen phosphate, lactate, HEPES, MOPS, BES, TES, MES, and Bis-Tris.

42. The method of any of claims 37 to 41, wherein the at least one inorganic salt comprises at least one of $CaCl_2$, KCl, $MgCl_2$, $MgSO_4$, NaCl, $NaHCO_3$, Na_2HPO_4, NaH_2PO_4 and ferric citrate chelate, or ferrous sulfate chelate.

43. The method of any of claims 37 to 42, wherein the at least one trace metal other than copper comprises ions of at least one of barium, bromium, cobalt, iodine, manganese, chromium, nickel, selenium, vanadium, titanium, germanium, molybdenum, silicon, iron, fluorine, silver, rubidium, tin, zirconium, cadmium, zinc and aluminium.

44. The method of any of claims 37 to 43, wherein the at least one polyamine comprises at least one of putrescine, cadaverine, spermine, and spermidine.

45. The method of any of claims 37 to 44, wherein the at least one essential fatty acid is selected from linoleic acid and linolenic acid.

46. A method of culturing a cell in a medium, the method comprising:

preventing loss of copper in the medium according to the method of any of

claims 1 to 25, or preparing the medium according to the method of any of claims 26 to 45,

contacting the cell with the medium, and

culturing the cell in the medium.

47. The method of claim 46, wherein the cell is a eukaryotic, a prokaryotic or an archaea cell.

48. The method of claims 47, wherein the eukaryotic cell is a mammalian cell.

49. The method of claim 48, wherein the mammalian cell is selected from hybridoma cells, CHO cells, COS cells, VERO cells, HeLa cells, HEK 293 cells, PER-C6 cells, K562 cells, MOLT-4 cells, Ml cells, NS-1 cells, COS-7 cells, MDBK cells, MDCK cells, MRC-5 cells, WI-38 cells, WEHI cells, SP2/0 cells, BHK cells (including BHK-21 cells) and derivatives thereof.

50. The method of any of claims 46 to 49, where the culturing the cell in the medium comprises multiplying the cell.

51. A method of making a biological product, comprising:

preventing loss of copper in the medium according to the method of any of claims 1 to 25 or preparing a medium according to the method of any of claims 26 to 45,

contacting a cell configured to express said biological product with the medium, and

culturing the cell in the medium under conditions such that the cell expresses the biological product.

52. The method of claim 51, wherein the biological product is a polypeptide, protein, peptide, hormone, virus or virus like particle, nucleic acid or a fragment thereof; optionally an antibody, or a biologically functional fragment of an antibody.

53. The method of claim 51 or 52, further comprising isolating and/or purifying the biological product.