LAYERED CELL CULTURE PARTICLES AND METHODS OF MAKING THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application no. 62/272,828, filed 30 December, 2015. The entire content of the aforementioned application is incorporated by reference herein.

FIELD OF THE INVENTION

[0002] The present invention relates to dry cell culture media, feeds, supplements, or concentrates comprising layered particles useful in culturing cells and other such applications. The invention relates to processes for preparing such layered compositions and methods of making cell culture compositions that are stable, for e.g., thermally, photo-chemically and/or to gamma irradiation. The invention also relates to the use of such layered particle preparations to produce proteins and polypeptides, and to increase cell growth and protein titers thereby.

SUMMARY

[0002a] In one aspect, the present disclosure refers to a method of making a media or a feed composition for culturing cells, comprising: i) subjecting a dry powder to suspension in a moving column of a gas in a fluid bed apparatus; ii) introducing at least one solvent using a sprayer into the dry powder of step (i) to form a layered base particle; iii) drying the layered base particle, wherein each solvent has a mixture of components and wherein the components are segregated into reactive species and into sensitive species that are sprayed separately to give a layered granule; wherein the reactive species are components that easily react with other components in the media or feed, thereby producing adducts, cross-linked products or precipitates; and wherein the sensitive species are components that may be destroyed by unwanted reaction with reactive species.

[0002b] In another aspect, the present disclosure refers to a method of making a media or a feed composition for culturing cells, comprising: i) subjecting a dry powder to suspension in a moving column of a gas in a fluid bed apparatus; ii) introducing a first solvent using a first sprayer onto the dry powder of step (i) to form a base granule; iii) introducing at least one second solvent using a second sprayer onto the base granule of step (ii) to form a layered base granule; iv) drying the layered base granule, wherein each solvent has a mixture of components and wherein the components are segregated into reactive species and

into sensitive species that are sprayed separately to give a layered granule; wherein the reactive species are components that easily react with other components in the media or feed, thereby producing adducts, cross-linked products or precipitates; and wherein the sensitive species are components that may be destroyed by unwanted reaction with reactive species.

[0002c] In another aspect, the present disclosure refers to a layered base particle or a layered base granule comprising in its core a mixture of sensitive components, anti-oxidant components, and neutral components, wherein said sensitive components, anti-oxidant components, and neutral components are layered one by one on top of each other, and wherein said sensitive components or said neutral components or said anti-oxidant components form the central core of the layered base granule, wherein the sensitive components are components that may be destroyed by unwanted reaction with reactive species.

[0002d] In yet another aspect, the present disclosure refers to a method of culturing a cell in a liquid reconstituted from the layered base particle or layered base granule as disclosed herein, comprising: i) reconstituting the layered base particle or layered base granule in a suitable liquid or buffer; wherein said layered base particle or layered base granule is a cell culture medium, feed, supplement or concentrate; ii) culturing the cell is the reconstituted liquid under conditions favorable for the growth of the cell.
[0002e] In yet another aspect, the present disclosure refers to a kit comprising: i) a first container comprising a layered base particle or a layered base granule as disclosed herein; wherein said layered base particle or said layered base granule is a cell culture medium, feed, supplement or concentrate; ii) instructions for using the layered base particle or the layered base granule for cell culture.

[0002e] In yet another aspect, the present disclosure refers to a system comprising: a liquid medium reconstituted from the layered base particle as disclosed herein and a cell.

[0003] In one example, the disclosure is directed in part to methods of making a media or a feed composition for culturing cells, comprising: i) subjecting a dry powder to suspension in a moving column of a gas in a fluid bed apparatus; ii) introducing at least one solvent using a sprayer into the dry powder of step (i) to form a layered base particle; and iii) drying the layered base particle.

[0004] In another example, the disclosure is also directed in part to methods of making a media or a feed composition for culturing cells, comprising: i) subjecting a dry powder to suspension in a moving column of a gas in a fluid bed apparatus; ii) introducing a first solvent using a first sprayer onto

the dry powder of step (i) to form a base granule; iii) introducing at least one second solvent using a second sprayer onto the base granule of step (ii) to form a layered base granule; iv) drying the layered base granule.

[0005] Also disclosed is that the dry powder may be a basal media powder, a complete media powder, a cell culture feed, a cell culture supplement, a cell culture media or feed concentrate or an amino acid mixture.

[0006] Also disclosed is that the first sprayer may be selected from the group consisting of: a top sprayer, a bottom sprayer and a tangential sprayer. Alternately, the first or the second sprayer may be selected from

[0015] Also disclosed is that the base particle or the base granule may be dried to a moisture content of about 0.5% to 10%.

[0016] Also disclosed is that the solvent additionally comprises at least one antioxidant, or at least one neutral species, or both.

[0017] Also disclosed is that the layered base particle or the layered base granule is obtained through any of the methods described above.

[0018] Also disclosed is that the methods are directed to producing a nutritive medium layered particle, a nutritive medium supplement layered particle, a nutritive medium subgroup layered particle, the method comprising making a layered media particle according to any of the methods described above and wherein at least one layer on the base particle or base granule comprises a reactive species and/or at least one layer on the base particle or base granule comprises a sensitive species. Also disclosed is that the reactive species may be selected from the group consisting of one or more trace elements, one or more reactive amino acids, one or more reactive amino acids, one or more metal salts, one or more acid soluble reactive groups, one or more alcohol soluble reactive groups and one or more pH modifier groups. Alternately, the sensitive species may be selected from the group consisting of one or more polyamines, one or more vitamins, one or more growth factor and one or more labile amino acids. In some examples, the dry powder may be selected from the group consisting of vitamins, bulk inorganic salts and sugars.

[0019] Also disclosed is that the methods are directed to culturing a cell in a liquid reconstituted from the layered media made as described above, the method comprising: i) reconstituting the layered media particle in a suitable liquid or buffer; wherein said layered media particle may be a cell culture medium, feed, supplement or concentrate; and ii) culturing the cell may be the reconstituted liquid under conditions favorable for the growth of the cell.

Also disclosed is that the culturing of the cell may be used to produce increased amount of a polypeptide. Also disclosed is that the polypeptide may be a recombinant polypeptide. Alternately, the culturing increases product production, increasing cell growth, as compared to a culture with liquid media not made from the layered media particles.

[0020] In yet another example, the present disclosure provides a kit comprising: i) a first container comprising a layered particle or granule according any one of the above claims; wherein said layered particle or granule may be a cell culture medium, feed, supplement or concentrate; and ii) instructions for using the layered particle or granule for cell culture.

[0021] In yet another example, the present disclosure provides a system comprising: a liquid medium reconstituted from a layered media particles according to the methods described above, and a cell. Also disclosed is that the reconstituted liquid medium may be used to cultivate a cell that produces a recombinant polypeptide, a virus, a secreted protein, or to cultivate a cell in suspension or a cell that may be attached.

[0022] Also disclosed are methods of making a media or a feed composition for culturing cells as described above, or, a kit as described above, or, a system as described above, wherein amino acids are used and the amino acids are selected from one or more of the twenty amino acids, their salts or derivatives thereof. Also disclosed is that the amino acids are selected from one or more of glycine, alanine, arginine, aspartic acid, glutamic acid, histidine, isoleucine, methionine, phenylalanine, proline, hydroxyproline, serine, threonine, tryptophan, valine, tyrosine, cysteine and lysine.

[0023] Also disclosed are layered particles or granules obtained through the methods described above used for culturing a cell. Also disclosed is that the cell may be a mammalian cell. Also disclosed is that the cell may be selected from the group consisting of CHO, BHK, HEK, 293, and VERO cells. Also disclosed is that the cell may be a plant cell, an animal cell, a eukaryotic cell, a eukaryotic cell, an algal cell, a fungal cell, and a bacterial cell.

INCORPORATION BY REFERENCE

[0024] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

CLAIMS

WHAT IS CLAIMED IS:

1. A method of making a media or a feed composition for culturing cells, comprising:

i) subjecting a dry powder to suspension in a moving column of a gas in a fluid bed apparatus;

ii) introducing at least one solvent using a sprayer into the dry powder of step (i) to form a layered base particle;

iii) drying the layered base particle, wherein each solvent has a mixture of components and wherein the components are segregated into reactive species and into sensitive species that are sprayed separately to give a layered granule;

wherein the reactive species are components that easily react with other components in the media or feed, thereby producing adducts, cross-linked products or precipitates; and

wherein the sensitive species are components that may be destroyed by unwanted reaction with reactive species.

2. A method of making a media or a feed composition for culturing cells, comprising:

i) subjecting a dry powder to suspension in a moving column of a gas in a fluid bed apparatus;

ii) introducing a first solvent using a first sprayer onto the dry powder of step (i) to form a base granule;

iii) introducing at least one second solvent using a second sprayer onto the base granule of step (ii) to form a layered base granule;

iv) drying the layered base granule, wherein each solvent has a mixture of components and wherein the components are segregated into reactive species and into sensitive species that are sprayed separately to give a layered granule;

wherein the reactive species are components that easily react with other components in the media or feed, thereby producing adducts, cross-linked products or precipitates; and

wherein the sensitive species are components that may be destroyed by unwanted reaction with reactive species.

3. The method of making the media or the feed composition for culturing cells of claim 1 or 2, wherein the dry powder is a basal media powder, a complete media powder, a cell culture feed, a cell culture supplement, a cell culture media or feed concentrate or an amino acid mixture.

4. The method of making the media or the feed composition for culturing cells of claim 1, wherein the first sprayer is selected from the group consisting of: a top sprayer, a bottom sprayer and a tangential sprayer.

5. The method of making the media or the feed composition for culturing cells of claim 2 or 3, wherein the first or the second sprayer is selected from the group consisting of: a top sprayer, a bottom sprayer and a tangential sprayer.

6. The method of making the media or the feed composition for culturing cells of claim 2 or 3 or 5, wherein the second sprayer is a bottom sprayer.

7. The method of making the media or the feed composition for culturing cells of any of claims 2 to 3 and claims5 to 6, wherein the second sprayer sprays a plurality of solvents on to the base granule to form a multilayered base granule.

8. The method of making the media or the feed composition for culturing cells of any of claims 1 or 3 to 4, wherein the second sprayer sprays a plurality of solvents on to the base particle to form a multilayered base particle.

9. The method of making the media or the feed composition for culturing cells of any of the preceding claims, wherein the second sprayer sprays the plurality of solvents sequentially, with intermittent drying steps between each spraying step.

10. The method of making the media or the feed composition for culturing cells of any of the preceding claims, wherein the second sprayer sprays the plurality of solvents sequentially, without any intermittent drying steps between each spraying step.

11. The method of making the media or the feed composition for culturing cells of any of the preceding claims, wherein each solvent in the plurality of solvents has one component in each solvent.

12. The method of making the media or the feed composition for culturing cells of claim 11, wherein each solvent in the plurality of solvents has the same components.

13. The method of making the media or the feed composition for culturing cells of claim 11, wherein each solvent in the plurality of solvents has different components.

14. The method of any of the preceding claims, wherein said reactive species is selected from the group consisting of one or more trace elements, one or more reactive amino acids, one or more reactive amino acids, one or more metal salts, one or more acid soluble reactive groups, one or more alcohol soluble reactive groups and one or more pH modifier groups.

15. The method of any of the preceding claims, wherein said sensitive species is selected from the group consisting of one or more polyamines, one or more vitamins, one or more growth factor and one or more labile amino acids.

16. The method of making the media or the feed composition for culturing cells of any of the preceding claims, wherein an inlet gas used in the method is at about temperature 20° C to 30° C.

17. The method of making the media or the feed composition for culturing cells of any of the preceding claims, wherein an inlet gas used in the method is at about temperature 25° C.

18. The method of making the media or the feed composition for culturing cells of any of the preceding claims, wherein the vitamins are selected from the group consisting of: vitamin B12, biotin, choline, folic acid, niacinamide, pyridoxine, riboflavin, thiamine, ascorbic acid and para-aminobenzoic acid (PABA).

19. The method of making the media or the feed composition for culturing cells of any of the preceding claims, wherein the drying step is at about temperature 50° C to 60° C.

20. The method of making the media or the feed composition for culturing cells of any of the preceding claims, wherein the base particle or the base granule is dried to a moisture content of about 0.5% to 10%.

21. The method of making the media or the feed composition for culturing cells of any of the preceding claims of any preceding claim, wherein the solvent comprises a reactive species.

22. The method of making the media or the feed composition for culturing cells of any of the preceding claims of any preceding claim, wherein the solvent additionally comprises at least one antioxidant.

23. A layered base particle or a layered base granule comprising in its core a mixture of sensitive components, anti-oxidant components, and neutral components, wherein said sensitive components, anti-oxidant components, and neutral components are layered one by one on top of each other, and wherein said sensitive components or said neutral components or said anti-oxidant components form the central core of the layered base granule, wherein the sensitive components are components that may be destroyed by unwanted reaction with reactive species.

24. The method according to any of the claims 1 to 22, wherein at least one layer on the layered base particle or layered base granule comprises a reactive species and/or at least one layer on the base particle or base granule comprises a sensitive species.

25. The method of any one of claims 1 to 22 and 24, wherein said reactive species is selected from the group consisting of one or more trace elements, one or more reactive amino acids, one or more reactive amino acids, one or more metal salts, one or more acid soluble reactive groups, one or more alcohol soluble reactive groups and one or more pH modifier groups.

26. The method of claim 24, wherein said sensitive species is selected from the group consisting of one or more polyamines, one or more vitamins, one or more growth factor and one or more labile amino acids.

27. The method of any one claims 1 to 22 and 24 to 26, wherein the dry powder is selected from the group consisting of vitamins, bulk inorganic salts and sugars.

28. A method of culturing a cell in a liquid reconstituted from the layered base particle or layered base granule of claim 23, comprising:

i) reconstituting the layered base particle or layered base granule in a suitable liquid or buffer; wherein said layered base particle or layered base granule is a cell culture medium, feed, supplement or concentrate;

ii) culturing the cell is the reconstituted liquid under conditions favorable for the growth of the cell.

29. The method of culturing the cell of claim 28, wherein the culturing is used to produce increased amount of a polypeptide.

30. The method of culturing the cell of claims 28 to 29, wherein the polypeptide is a recombinant polypeptide.

31. The method of culturing the cell of claims 28 to 30, wherein the culturing increases product production, increasing cell growth, as compared to a culture with liquid media not made from the layered media particles.

32. A kit comprising:

i) a first container comprising a layered base particle or a layered base granule according to claim 23; wherein said layered base particle or said layered base granule is a cell culture medium, feed, supplement or concentrate;

ii) instructions for using the layered base particle or the layered base granule for cell culture.

33. A system comprising: a liquid medium reconstituted from the layered base particle according to claim 23 and a cell.

34. The system of claim 33, wherein the reconstituted liquid medium is used to cultivate a cell that produces a recombinant polypeptide, a virus, a secreted protein, or to cultivate a cell in suspension or a cell that is attached.

35. The making a media or a feed composition for culturing cells of any of the above claims, or, the kit of claim 32, or, the system of claims 33-34, wherein the amino acids are selected from one or more of the twenty amino acids, their salts or derivatives thereof.

36. The making a media or a feed composition for culturing cells of any of the above claims, or, the kit of claim 32, or, the system of claims 33-34, wherein the amino acids are selected one or more of glycine, alanine, arginine, aspartic acid, glutamic acid, histidine, isoleucine, methionine, phenylalanine, proline, hydroxyproline, serine, threonine, tryptophan, valine, tyrosine, cysteine and lysine.

37. The system of claim 33, wherein the cell is a mammalian cell.

38. The system of claim 37, wherein the cell is selected from the group consisting of CHO, BHK, HEK, 293, and VERO cells.

39. The system of claim 33, wherein the cell is a plant cell, an animal cell, a eukaryotic cell, an algal cell, a fungal cell, and a bacterial cell.