

WHAT IS CLAIMED IS:

1. A method of perfusion culturing a mammalian cell, the method comprising:
providing a vessel containing a mammalian cell disposed in a first liquid culture medium having an osmolality of 270 mOsm/kg to 380 mOsm/kg;
incubating the mammalian cell for a period of time at 32 °C to 39 °C;
during the period of time, continuously or periodically removing a first volume of the first liquid culture medium and adding to the first liquid culture medium a second volume of a second liquid culture medium; and
during the period of time, continuously monitoring the conductivity of the first and second liquid culture medium in the vessel using an online conductivity sensor;
wherein:
the first and second volumes are equal and the osmolality of the first and second liquid culture medium in the vessel is maintained at 270 mOsm/kg to 380 mOsm/kg over the period of time by addition of a salt solution controlled by the online conductivity sensor.
2. The method of claim 1, wherein the first liquid culture medium has an osmolality of 270 mOsm/kg to 320 mOsm/kg.
3. The method of claim 1, wherein the second liquid culture medium comprises greater than 8 g/L of a poloxamer.
4. The method of claim 3, wherein the poloxamer is Pluronic F68.
5. The method of any one of claims 1-4, wherein the salt solution comprises sodium chloride.
6. The method of any one of claims 1-5, wherein maintaining the osmolality of the first and second liquid culture medium in the vessel further comprises adjusting the flow rate of the second liquid culture medium at some point during the period of time.

7. The method of claim 6, wherein the adjusting step comprises increasing the flow rate of the second liquid culture medium at some point during the period of time.

8. The method of claim 7, wherein the method further comprises adjusting one or both of the pH and the pCO₂ of the first and second liquid culture medium in the vessel at some point during the period of time.

9. The method of claim 8, wherein the adjusting step comprises decreasing the flow rate of the second liquid culture medium at some point during the period of time.

10. The method of any one of claims 1-9, wherein the method further comprises increasing the pCO₂ and increasing the pH of the first and the second liquid culture medium in the vessel over the period of time.

11. The method of claim 10, wherein the pCO₂ and the pH of the first and the second liquid culture medium in the vessel are increased based on the viable cell density over the period of time.

12. The method of any one of claims 1-11, wherein a viable cell density of greater than 60×10^6 cells/mL in the first and second liquid culture medium is achieved during the period of time.

13. The method of any one of claims 1-12, wherein the vessel is a bioreactor.

14. The method of any one of claims 1-13, wherein the mammalian cell contains a nucleic acid encoding a recombinant protein.

15. The method of claim 14, wherein the method further comprises:
recovering the recombinant protein from the mammalian cell or from the first and/or second liquid culture medium.

16. The method of claim 15, wherein the method further comprises isolating the recombinant protein.

17. The method of claim 16, wherein the method further comprises formulating the isolated recombinant protein.

18. The method of any one of claims 1-17, wherein the mammalian cell is a CHO cell.