

CLAIMS

1. Method for continuous recovering of a protein from a fluid, wherein the method comprises the following steps:

a protein precipitation step of precipitating the protein in the fluid; and

a protein separation step of separating the precipitated protein from the fluid;

wherein all steps are performed in an integrated process.
2. The method of claim 1, wherein all steps of the method are performed continuously.
3. The method of claim 1 or claim 2, wherein the protein has a molecular weight of 250 kDa or more, preferably wherein the protein has a molecular weight of 500 kDa or more.
4. The method of any one of claims 1 to 3, wherein before the protein precipitation step the concentration of the protein in the fluid comprising the protein is below 20 µg/ml, preferably between 0.05 µg/ml and 20 µg/ml.
5. The method of any one of claims 1 to 4, wherein the protein is a blood coagulation factor.
6. The method of claim 5, wherein the protein is Factor VIII.
7. The method of any one of claims 1 to 4, wherein the protein is von Willebrand factor.
8. The method of any one of claims 1 to 4, wherein the protein is a protein complex comprising Factor VIII and von Willebrand factor.
9. The method of any one of claims 1 to 8, wherein in the protein precipitation step the protein is precipitated using a precipitant.
10. The method of claim 9, wherein the precipitant is selected from the group consisting of calcium phosphate, polyethylene glycol (PEG), an affinity ligand, a pH modifying agent, an organic solvent such as ethanol or acetone, a polyelectrolyte such as polyacrylic acid or polyethylenimine, and a salt.
11. The method of claim 9 or 10, wherein the precipitant comprises phosphate.
12. The method of any one of claims 9 to 11, wherein the precipitant is calcium phosphate, magnesium phosphate, or zinc phosphate.
13. The method of any one of claims 9 to 12, wherein the precipitant is calcium phosphate.
14. The method of claim 13, wherein the protein precipitation step comprises adding calcium ions to the fluid.

15. The method of claim 14, wherein calcium ions are added to a final concentration of between 10 mM and 50 mM, preferably between 10 mM and 30 mM.
16. The method of claim 14, wherein calcium ions are added to a final concentration of between 10 mM and 20 mM, preferably about 15 mM.
17. The method of any one of claims 13 to 16, wherein the protein precipitation step comprises adding phosphate ions to the fluid.
18. The method of claim 17, wherein phosphate ions are added to a final concentration of between 1 mM and 10 mM, preferably between 1 mM and 5 mM.
19. The method of claim 17, wherein phosphate ions are added to a final concentration of between 1 mM and 3 mM, preferably about 2 mM.
20. The method of any one of claims 9 to 19, wherein the protein precipitation step comprises mixing the fluid comprising the protein and the precipitant.
21. The method of claim 20, wherein mixing is performed in at least one reactor selected from the list consisting of a continuous stirred tank reactor (CSTR), a tubular reactor (TR), a segmented flow reactor, and an impinging jet reactor.
22. The method of claim 20 or 21, wherein mixing is performed in a continuous stirred tank reactor (CSTR).
23. The method of any one of claims 1 to 22, wherein the pH of the fluid before precipitating the protein is adjusted to a pH of between 8.5 and 9.0, preferably to a pH of about 8.75.
24. The method of any one of claims 1 to 23, wherein the pH of the fluid after precipitating the protein is between 6 and 7.5, preferably between 6.5 and 7, most preferably about 6.5.
25. The method of any one of claim 1 to 24, wherein in the protein separation step a plate settler for protein separation, continuous tangential flow filtration, or fluidized bed centrifugation is used for separating the precipitated protein from the fluid.
26. The method of any one of claims 1 to 25, wherein in the protein separation step a plate settler for protein separation is used for separating the precipitated protein from the fluid.
27. The method of claim 26, wherein the plate settler for protein separation is an inclined plate settler with a lower portion, an upper portion, and at least one sedimentation channel for letting the precipitated protein settle, said sedimentation channel extend from the lower portion to the upper portion,

the inclined plate settler being configured to be oriented during use such that the at least one sedimentation channel extends from the lower portion to the upper portion in a direction that is inclined with respect to the direction of gravity,

wherein the at least one sedimentation channel is connected to a fluid outlet for draining a rest fluid at the upper portion.

28. The method of claim 27, wherein the length of the sedimentation channel is between 20 cm and 150 cm, preferably between 20 cm and 100 cm, more preferably between 20 cm and 80 cm, more preferably between 30 cm and 70 cm, more preferably between 40 cm and 60 cm, most preferably about 50 cm.

29. The method of claim 27 or 28, wherein the at least one sedimentation channel of the plate settler for protein separation is connected to a bottom section, wherein the bottom section comprises at least one inlet channel for feeding the fluid comprising the precipitated protein to the plate settler, and at least one collection channel for collecting the settled precipitated protein descending from the at least one sedimentation channel,

wherein said at least one inlet channel and said at least one collection channel are fluidly separated from each other, said inlet channel and said collection channel being connected to said at least one sedimentation channel, to form fluid connections between said at least one inlet channel and said at least one sedimentation channel and between said at least one collection channel and said at least one sedimentation channel, respectively.

30. The method of claim 29, wherein the bottom section that is connected to the plate settler for protein separation further comprises at least one wash fluid supply channel for supplying a wash fluid to one sedimentation channel or to one collection channel, said at least one wash fluid supply channel being fluidly separated from other wash fluid supply channels and from all inlet channels.

31. The method of claim 30, wherein the at least one wash fluid supply channel and the at least one collection channel corresponding to the same sedimentation channel of the plate settler for protein separation are fluidly connected by an opening in a wall portion shared by said wash fluid supply channel and said collection channel.

32. The method of claim 30 or claim 31, wherein the fluid comprising the precipitated protein is supplied to the bottom section, which is connected to the plate settler for protein separation, through the at least one inlet channel, and a wash fluid is supplied through the at least one wash fluid supply channel,

wherein the density of the wash fluid is higher than the density of the fluid comprising the precipitated protein, and

wherein the rest fluid is drained through the fluid outlet at the upper portion and the settled precipitated protein is drained through the collection channel.

33. The method of claim 32, wherein the density of the wash fluid is between 0.3 ‰ and 1.5 ‰ higher than the density of the fluid comprising the precipitated protein, preferably between 0.55 ‰ and 1.20 ‰ higher than the density of the fluid comprising the precipitated protein.

34. The method of claim 32 or 33, wherein the wash fluid comprises Tris and sodium chloride.

35. The method of claim 34, wherein the wash fluid comprises Tris at a concentration of about 2 mM and sodium chloride at a concentration of about 272 mM.
36. The method of claim 34 or 35, wherein the wash fluid further comprises calcium chloride.
37. The method of claim 36, wherein the wash fluid comprises calcium chloride at a concentration of between 4 mM and 12 mM.
38. The method of claim 36 or 37, wherein the wash fluid comprises Tris at a concentration of about 2 mM, sodium chloride at a concentration of about 231 mM and calcium chloride at a concentration of about 12 mM.
39. The method of any one of claims 32 to 38, wherein the wash fluid has a pH of 7.5 or higher, preferably of 8 or higher, most preferably of about 8.25.
40. The method of any one of claims 32 to 39, wherein the wash fluid is supplied through the at least one wash fluid supply channel and the settled precipitated protein is drained through the collection channel at regular intervals.
41. The method of claim 40, wherein the wash fluid is supplied through the at least one wash fluid supply channel and the settled precipitated protein is drained through the collection channel at regular intervals of between 15 min and 45 min, preferably about 30 min.
42. The method of any one of claims 32 to 41, wherein the wash fluid is supplied through the at least one wash fluid supply channel and the settled precipitated protein is drained through the collection channel at a volumetric flow rate of about 20 to 60 mL/min, preferably about 40 mL/min.
43. The method of any one of claims 1 to 42, wherein the method further comprises the following steps before the protein precipitation step:
 - a protein production step of culturing cells in a fluid, wherein the cells produce the protein and release the protein into the fluid; and
 - a cell separation step of separating the cells from the fluid comprising the protein.
44. The method of claim 43, wherein the cells are mammalian cells.
45. The method of claim 44, wherein the cells are CHO cells.
46. The method of any one of claims 43 to 45, wherein the fluid is a cell culture medium.
47. The method of any one of claims 43 to 46, wherein in the protein production step the cells are cultured in a perfusion reactor or a chemostat reactor, preferably in a chemostat reactor.

48. The method of any one of claims 43 to 47, wherein in the cell separation step a plate settler for cell separation is used for separating the cells from the fluid comprising the protein.
49. The method of claim 48, wherein the plate settler for cell separation is an inclined plate settler with a lower portion, an upper portion, and at least one sedimentation channel for letting the cells settle, said sedimentation channel extend from the lower portion to the upper portion,

the inclined plate settler being configured to be oriented during use such that the at least one sedimentation channel extends from the lower portion to the upper portion in a direction that is inclined with respect to the direction of gravity,

wherein the at least one sedimentation channel is connected to a fluid outlet for draining a rest fluid at the upper portion.

50. The method of claim 49, wherein the at least one sedimentation channel of the plate settler for cell separation is connected to a bottom section, wherein the bottom section comprises at least one inlet channel for feeding the fluid comprising the cells and the protein to the plate settler, and at least one collection channel for collecting the settled cells descending from the at least one sedimentation channel,

wherein said at least one inlet channel and said at least one collection channel are fluidly separated from each other, said inlet channel and said collection channel being connected to said at least one sedimentation channel, to form fluid connections between said at least one inlet channel and said at least one sedimentation channel and between said at least one collection channel and said at least one sedimentation channel, respectively.

51. The method of claim 50, wherein the bottom section that is connected to the plate settler for cell separation further comprises at least one wash fluid supply channel for supplying a wash fluid to one sedimentation channel or to one collection channel, said at least one wash fluid supply channel being fluidly separated from other wash fluid supply channels and from all inlet channels.
52. The method of claim 51, wherein the at least one wash fluid supply channel and the at least one collection channel corresponding to the same sedimentation channel of the plate settler for cell separation are fluidly connected by an opening in a wall portion shared by said wash fluid supply channel and said collection channel.
53. The method of claim 51 or 52, wherein the fluid comprising the cells and the protein is supplied to the bottom section, which is connected to the plate settler for cell separation, through the at least one inlet channel, and a wash fluid is supplied through the at least one wash fluid supply channel,

wherein the density of the wash fluid is higher than the density of the fluid comprising the cells and the protein, and

wherein the settled cells are drained through the collection channel and the rest fluid comprising the protein is drained through the fluid outlet at the upper portion.

54. The method of claim 53, wherein the wash fluid is supplied through the at least one wash fluid supply channel and the settled cells are drained through the collection channel at regular intervals.
55. The method of claim 53 or 54, wherein the wash fluid is supplied through the at least one wash fluid supply channel and the settled cells are drained through the collection channel at regular intervals of 5 min to 90 min.
56. The method of claim 53 or 54, wherein the wash fluid is supplied through the at least one wash fluid supply channel and the settled cells are drained through the collection channel at regular intervals of 15 min to 85 min.
57. The method of claim 53 or 54, wherein the wash fluid is supplied through the at least one wash fluid supply channel and the settled cells are drained through the collection channel at regular intervals of 25 min to 80 min.
58. The method of claim 53 or 54, wherein the wash fluid is supplied through the at least one wash fluid supply channel and the settled cells are drained through the collection channel at regular intervals of 35 min to 75 min.
59. The method of claim 53 or 54, wherein the wash fluid is supplied through the at least one wash fluid supply channel and the settled cells are drained through the collection channel at regular intervals of 45 min to 70 min.
60. The method of claim 53 or 54, wherein the wash fluid is supplied through the at least one wash fluid supply channel and the settled cells are drained through the collection channel at regular intervals of 55 min to 65 min.
61. The method of claim 53 or 54, wherein the wash fluid is supplied through the at least one wash fluid supply channel and the settled cells are drained through the collection channel at regular intervals of about 60 min.
62. The method of any one of claims 53 to 61, wherein the wash fluid is supplied through the at least one wash fluid supply channel and the settled cells are drained through the collection channel at a volumetric flow rate of between 50 to 70 mL/min, preferably about 60 mL/min.
63. The method of any one of claims 1 to 62, wherein the method further comprises the following step after the protein separation step:

a re-solubilization step of re-solubilizing the precipitated protein.
64. The method of claim 63, wherein in the re-solubilization step the precipitated protein is re-solubilized using citrate or EDTA.
65. The method of claim 64, wherein in the re-solubilization step the precipitated protein is re-solubilized using EDTA.

66. The method of claim 65, wherein in the re-solubilization step the precipitated protein is re-solubilized using EDTA at a final concentration of between 10 mM to 50 mM.
67. The method of claim 65 or 66, wherein in the re-solubilization step the precipitated protein is re-solubilized using EDTA at a final concentration of between 20 mM to 30 mM.
68. The method of any one of claims 65 to 67, wherein in the re-solubilization step the precipitated protein is re-solubilized using EDTA at a final concentration of about 25 mM.
69. The method of any one of claims 1 to 68, wherein the protein is a biopharmaceutical drug.
70. Recovered protein that is obtainable by the method of any one of claims 1 to 69.
71. Method for producing a pharmaceutical composition, comprising performing the method of claim 69 and formulating the recovered biopharmaceutical drug as a pharmaceutical composition.
72. Pharmaceutical composition that is obtainable by the method of claim 71.
73. An inclined plate settler for separating a solid component from a fluid, wherein the plate settler comprises a lower portion, an upper portion, and at least one sedimentation channel for letting the solid component settle, said sedimentation channel extend from the lower portion to the upper portion,
- the plate settler being configured to be oriented during use such that the at least one sedimentation channel extends from the lower portion to the upper portion in a direction that is inclined with respect to the direction of gravity,
- wherein the at least one sedimentation channel is connected to a fluid outlet for draining a rest fluid at the upper portion and connected to a bottom section at the lower portion,
- wherein the bottom section comprises at least one inlet channel for feeding a fluid comprising the solid component to be separated to the plate settler, and at least one collection channel for collecting a settled component descending from the at least one sedimentation channel,
- wherein said at least one inlet channel and said at least one collection channel are fluidly separated from each other, said inlet channel and said collection channel being connected to said at least one sedimentation channel, to form fluid connections between said at least one inlet channel and said at least one sedimentation channel and between said at least one collection channel and said at least one sedimentation channel, respectively.
- wherein the bottom section further comprises at least one wash fluid supply channel for supplying a wash fluid to one sedimentation channel or to one collection channel, said at least one wash fluid supply channel being fluidly separated from other wash fluid supply channels and from all inlet channels, and

wherein the length of the sedimentation channel is between 20 cm and 150 cm, preferably between 20 cm and 100 cm, more preferably between 20 cm and 80 cm, and most preferably between 30 cm and 70 cm.

74. The inclined plate settler of claim 73, wherein the length of the sedimentation channel is between 40 cm and 60 cm, preferably about 50 cm.
75. The inclined plate settler of claim 73 or 74, wherein the solid component is a precipitated protein, preferably a precipitated protein complex comprising Factor VIII and von Willebrand factor.
76. The inclined plate settler of any one of claims 73 to 75, wherein the inclined plate settler contains a precipitated protein complex comprising Factor VIII and von Willebrand factor.